STUDIES ON THE PHYSICAL PROPERTIES AND PRESERVATION OF THE NORTH AMERICAN PAWPAW (*Asimina triloba*) FRUIT

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by

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The undersigned, appointed by the dean of the Graduate School have examined the thesis entitled:

STUDIES ON THE PHYSICAL PROPERTIES AND PRESERVATION OF THE NORTH AMERICAN PAWPAW (*Asimina triloba*) FRUIT

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a candidate for the degree of Master of Science,

and hereby certify that, in their opinion, it is worthy of acceptance.

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DEDICATION

I dedicate this work to my parents and siblings whose prayers and support have been unparalleled throughout my career and academic pursuits.

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Glory to God for the divine strength and enablement He has given me through Jesus Christ that has brought me thus far.

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Abstract

The North American pawpaw (*Asimina triloba*) fruit is an annonaceous fruit that belongs in the same family as tropical fruits like soursop, cherimoya, atemoya, and others. Pawpaw is the only fruit in the Annonaceae family that grows in temperate regions of the world. The fruit is native to the United States and grows in over 30 states in the United States including Missouri, Indiana, Ohio, Illinois, and parts of Texas. The fruit has remained underutilized and uncommercialized for several centuries. The main factors that have contributed to it being underutilized and uncommercialized are the rapid changes in quality that occur in the fruit after it is harvested. The fruit loses firmness, and the color of the skin rapidly changes from yellowish green to brown or black within 3-5 days. This study aims to evaluate the physical characteristics of different cultivars of the pawpaw fruit to gain insights into its processing potential, test correlations between noninvasive parameters like skin color and invasive parameters like textural properties to establish noninvasive ripening indicators and compare the effect of edible coatings on the quality of pawpaw fruits during storage. The findings from the study show that among the cultivars studied, the Susquehanna cultivar has the highest fruit weight and pulp yield, while the Overleese cultivar has the highest juice content, making the Susquehanna cultivar potentially suitable for fruit processing operations that require high pulp yield and the Overleese cultivar potentially suitable for fruit juice processing. Also, all the cultivars studied had peel

thickness between 0.21 and 0.72mm, making them highly susceptible to bruising and eventual postharvest losses if they are not handled carefully. Further, analyses of the textural properties show that the ripe pawpaw fruits have a hardness of 2.2 ± 0.5 kg-force, similar to the hardness of green ripe mangoes but harder than green ripe bananas, and the unripe fruits have a hardness of 68.2 ± 10.9 kg-force. Although skin color had previously been thought to be a poor indicator of pawpaw fruit ripeness, the results from the correlations show that there are statistically significant strong negative correlations between the fruit skin color a* values (greenness) and hardness (r =−0.87), chewiness (r = −0.86), and cohesiveness ratio (r = −0.73), and a strong positive correlation with total soluble solids (r = 0.90). The skin hue angles also have strong positive correlations with hardness (r = 0.86), cohesiveness ratio (r = 0.74), and chewiness (r = 0.86), and a strongly negative correlation with total soluble solids (r = −0.91). Hence, instead of determining the ripeness of the fruit using hardness which is an invasive method, noninvasive parameters like the skin color greenness and hue angles can be used to determine pawpaw fruit ripeness. In addition, the results from the preservation studies show that chitosan coatings are more effective in slowing moisture loss in Sunflower fruits than in Susquehanna and 10-35 fruits over time. The TOPSIS-Shannon entropy analyses showed that the 10-35 fruits with 1% chitosan had the most stable quality over time, followed by the Susquehanna and Sunflower fruits with 2% chitosan coatings. The experimental data from different cultivars, treatments, and storage conditions proved the shelf-life of pawpaw fruit could be extended from 5 days to 15-20 days depending on the cultivar. These findings will enable the creation of markets for pawpaw fruits and allow countries that grow them to generate revenue from this underutilized specialty crop

Chapter 1

Introduction

The North American pawpaw (*Asimina triloba* [L.] Dunal) fruit, also known as poor man's banana, pawpaw, Indian banana, or dog banana, is a tropical fruit that grows in temperate regions of the world. The fruit belongs in the Annonaceae family with tropical fruits like cherimoya, soursop, sugar apple, atemoya, and others. The fruit is known to be the largest fruit native to the United States. In the United States, pawpaw grows in the temperate woodlands or mesic hardwood forests in USDA plant hardiness zones 5-8 (Pomper et al., 2008b). It grows in over 30 states (including Missouri, Indiana, Kentucky, Ohio, and North Carolina) in the United States, Canada, South Korea, Nigeria, and Romania. According to Pomper et al. (1999), pawpaw grows well in the deep, rich fertile soils of river-bottom lands where they grow as understory trees or thicket shrubs. The ornamental value of the pawpaw tree in addition to the insecticidal and anti-tumor compounds present in the leaves, bark, twigs, and fruit make it crucial to conduct studies to gain insights about the pawpaw.

The pawpaw fruit is oblong in shape with a thin inedible peel, edible pulp, and two rows of inedible, black seeds about the size of almonds. Generally, the skin of the fruit is green when the fruit is unripe and changes to greenish-yellow when the fruit is ripe and eventually to brown or black as it overripens. The season for harvesting the pawpaw fruit is between August and early October, depending on the cultivar and climatic conditions during the year preceding the harvest season.

Further, the pawpaw fruit has a unique set of characteristics that make the fruit one with great potential as a high-value specialty crop. The pawpaw fruit has an aroma/flavor that has often been described as a combination of banana, mango, and pineapple flavors. In addition to these aroma/flavor descriptors, others such as apple, melon, and fresh flavors have also been used by trained sensory panelists to describe the flavor of pawpaw fruits (Duffrin & Pomper, 2006; Pomper et al., 2010). Studies have shown that the volatile compounds that give the fruit its distinct flavor notes are mainly ethyl esters made of 50.2% hexanoate, 19.3% octanoate, 8.5% butanoate, and 1.3% decanoate, and methyl esters like butanoate, hexanoate, octanoate, geranate, decanoate, and fernesate (Shiota, 1991). Among all these flavor compounds, McGrath & Karahadian (1994) found that ethyl hexanoate was found to be present in the highest concentration in a variety of cultivars.

Nutritional analysis of the fruit pulp shows that pawpaw contains three times the amount of vitamin C in apples, twice the amount of riboflavin in oranges, fourteen times the amount of niacin in apples, and contains even more essential amino acids than apples (Jones & Layne, 1997). Chemical studies on the pawpaw plant have indicated that various parts of the pawpaw plant contain neurotoxins and anticancer compounds. These compounds can be found in the fruit pulp, seeds, twigs, bark, and leaves of the pawpaw plant. Studies by Potts et al. (2012) and Levine et al. (2015) indicate that the pawpaw fruit pulp contains acetogenins (annonacin and squamocin) which are toxic to cortical neurons in a concentration-dependent manner, hence, with chronic exposure to pawpaw products the effect of these acetogenins could increase the risk for neurodegeneration. Though some scientists have cautioned the consumption of pawpaw fruits, more recent studies by Nam et al. (2018) indicate that pawpaw pulp contains annonacin, asimin, aromin, cis-annonacin,

and annomuricin, which inhibit the growth of cancer cells, though the antiproliferative activity was found to be higher in unripe fruits as compared to ripe fruits.

Pawpaw (*Asimina triloba*) has remained an underutilized fruit for several centuries. In 1916, the American Genetics Association noted that the greatest barrier to developing a market for the fruit is its perishability (American Genetic Association, 1916). However, to date, harvest and storage techniques have not been developed for the pawpaw fruit (Archbold & Pomper, 2003). The perishability of the fruit is largely due to its rapid ripening after harvesting. Pawpaw is a climacteric fruit; hence, it continues to ripen after harvesting thereby losing firmness and becoming difficult to handle in 5 days (Galli et al., 2008). The respiration and ethylene gas production of the fruit have not been studied extensively to be able to understand the right conditions for storage and handling.

In addition, very little research has been done to further understand the fruit and develop appropriate storage and handling techniques to prolong its shelf life and make it more accessible to consumers. In a search on lens.org using the search terms “Asimina triloba”, the search results show that there are 421 scholarly articles on the fruit with a bulk of them focusing on the biology, botany, and horticulture of the fruit. Further search using the terms “Asimina triloba AND preservation” shows that only one scholarly article by Galli et al. (2008) studies how the shelf life of the whole fruit can be extended through cold storage. There have been other studies which have focused on various aspects of the quality of the pulp from the fruit using techniques like high pressure processing and how to utilize the pulp in food processing, aside the artisanal attempts to make various products like wine and jam from the pawpaw fruits (Brannan et al., 2019; Wiese & Duffrin, 2003; L. Zhang et al., 2017).

Many gaps in pawpaw research are yet to be filled. It is crucial to conduct further studies to understand the physical properties and develop techniques for extending the shelf life of pawpaw fruits to aid its commercialization. Hence, the main objective of this study was to investigate the physical properties and preservation of the North American pawpaw fruit. The specific objectives of this study were:

1. To investigate the physical characteristics of frozen fruits from eight cultivars of the North American pawpaw.
2. To test correlations between color, textural properties, and ripening of the North American pawpaw fruit.
3. To test the effect of edible coatings and freshness paper treatments on the quality of North American pawpaw fruits during storage.

Chapter 2

Literature Review

2.1 Pawpaw cultivation

The North American pawpaw is cultivated for ornamental purposes in parks and gardens, especially in butterfly gardens due to its attractive form and foliage and because it is the exclusive larval host plant for the zebra swallowtail butterfly (*Eurytides marcellus* Cramer) (H. Huang et al., 2003; Lolletti et al., 2021). According to Geneve et al. (2003), pawpaw is most often propagated from recalcitrant seeds and must be stored moist at a chilling temperature. However, fruit quality from seed propagation is variable and usually inferior to selected cultivars for cultivation in orchards. Hence, pawpaw cultivars with superior fruit characteristics are propagated by grafting onto seedling understocks. Further, pawpaw can be propagated from cuttings, but only in very young seedling stock plants.

2.2 Cultivars

Over the years, several cultivars of the pawpaw fruit have been selected for their excellent characteristics. According to Pomper et al. (2003) and Peterson (2003), at least 56 cultivars of pawpaw were selected and named between 1900 and 1960, however, less than 20 of these cultivar selections are remaining as many of the cultivars have been lost through neglect, abandonment of collections, and loss of records necessary for identification. Nonetheless, since 1960, horticulturalists have selected and developed additional pawpaw cultivars (Pomper et al., 2003). Currently, there are about 47 known pawpaw cultivars with unique properties and varying growth rates and ripening times that are being grown in different parts of the world (Pomper et al., 2009).

Pawpaw cultivars that are currently known include Susquehanna, Sunflower, Wabash, Overleese, NC-1, Lynn's Favorite and Potomac, which are known to bear large fruits, while Davis, Glaser, Prolific, Shenandoah, Taylor, PA-Golden and Wells are known to bear medium-sized fruits and Middletown, Rappahannock and Wilson cultivars which are known to bear small-sized fruits (Pomper et al., 2009). Further, some of the cultivars are hybrids of other cultivars. For example, Kirsten cultivar is a hybrid of Taytwo and Overleese cultivars, and IXL and NC-1 cultivars are hybrids of Overleese and Davis cultivars (Brannan et al., 2015; Pomper et al., 2009).

Beyond the differences in the sizes of the fruits from the different cultivars, some cultivars ripen earlier than others. Fruits from Allegheny and Shenandoah cultivars are known to ripen early in the harvest season, Rappahannock, and Tallahatchie (10-35) cultivars ripen in the middle of the harvest season, and fruits from Susquehanna, Wabash and Potomac ripen late in the harvest season (Moore, 2015; Pomper et al., 2009).

2.3 Indicators of pawpaw fruit ripeness

The use of objective indicators of ripeness is important for preventing postharvest loss of fruits (Porat et al., 2018). The identification and development of objective ripeness indicators can be done by first understanding the progression of ripening in the fruit. However, to date, only a few studies have sought to understand the ripening process of the pawpaw fruit and develop objective parameters for assessing the ripeness of the fruit.

In a study by Mcgrath & Karahadian (1994), some physical, chemical, and sensory properties of the pawpaw fruit were studied to assess how these properties can be used as indicators of pawpaw ripeness. In this study, it was found that as the fruits ripened, there was a sharp increase in the concentration of headspace volatile compounds coupled with

an increase in the intensity of fruity aroma and soluble solids content. In addition, they noted that as pawpaw fruits ripened, there was a decrease in hardness of the fruit with skin color hue angles less than 100º. Kobayashi et al. (2008) and Nam et al. (2019) also studied phenolic content and antioxidant capacity of pawpaw fruits at different stages of ripening in different cultivars found that as the fruits ripened, the phenolic content and antioxidant capacity decreased. However, the challenge with using these parameters to assess ripeness is that they are invasive, require expensive instruments and reagents, and may not be productive for pawpaw farmers.

Archbold & Pomper (2003) studied the ripening of the fruit as it relates to the respiration and release of ethylene gas. They noted that pawpaw fruits are climacteric fruits since they have a maximum respiration rate of 90mg kg^{-1} h^{-1} and ethylene production rate of 14.4µg kg^{-1} h^{-1}, although the peak ethylene production rate of pawpaw was considerably less than the values for cherimoya, atemoya and soursop which range from 50 to 300 µg kg^{-1} h^{-1}. Climacteric fruits are those whose ripening is associated to a peak of ethylene production and an increase in respiration rate (Chen et al., 2018), and a good understanding of the respiration rate and ethylene production during ripening can help to identify indicators for ripeness of fruits. However, in the study conducted by Archbold & Pomper (2003), the cultivar of pawpaw used in the study is not indicated, which makes it difficult to use as a point of reference since ripening of fruits is cultivar-dependent (Alós et al., 2019). In another study by Galli et al. (2008), fruits from the Wabash, Middletown, PA Golden, Taylor, Taytwo, Shenandoah, Wells, and Wilson, 8-20 and 9-58 cultivars harvested during the August−October harvest seasons of 2001, 2002, 2004, and 2005 were studied. However, fruits from multiple cultivars harvested in the 2001 and 2002 batches

were pooled together for the analyses and the fruits from the 2004 and 2005 batches had mold growth during the experiment and were then discarded, limiting the extent to which the data obtained in the study can be applied to provide ripeness indicators.

2.4 Physical properties of the North American pawpaw fruit

Unlike fruits like mangoes and bananas whose physical properties can be easily used to determine ripeness and assess the quality of the produce, various studies are being conducted to understand the physical properties of the North American pawpaw fruit as it relates to its ripeness and quality. Studies on the physical properties of fruits like weight, size, skin color, pulp color, and textural properties, among others provide valuable insights into the quality of the fruits, aid in the selection of processing equipment and processing conditions, and help plant breeders to develop cultivars with improved characteristics. A few studies have evaluated various physical characteristics of the pawpaw fruit. The physical characteristics that have been studied so far are discussed in the following sections.

2.5 Size of the North American pawpaw fruit

Fruit size is an important indicator of fruit maturity and pulp yield. According to Mauxion et al. (2021), fruit size is a complex trait that is the result of strict spatial and temporal control and coordination of overlapping and interconnected cellular events, cell division, and cell expansion, occurring with different onsets, rates, and duration. Studies have shown that pawpaw fruits are generally ovate in shape with weights going up to 1kg in size with varying shapes depending on the cultivar (Adainoo et al., 2022; Brannan et al., 2015). The fruit is 3-15 cm in length and 3-10 cm in width and contains 12-20 seeds that may each be 3 cm long (Brannan et al., 2015; Pomper et al., 2008b). While these studies

have measured various aspects of fruit size such as weight, length and width, the fruit size of Middletown, Overleese, Wells, Ithaca, Mary Foos Johnson, NC-1, Sunflower, Taylor, Wilson, and a few other cultivars have been documented in research publications (Brannan et al., 2015; Lolletti et al., 2021; Pomper et al., 2008a). It is crucial to assess the fruit size as an important property of fruits from the other cultivars that have been selected/developed. Findings on their fruit size will help processors to identify which cultivars with high pulp yield to use for efficient processing of the fruit.

2.6 Skin color and pulp color of the North American pawpaw fruit

The color of fruits is one of the main factors consumers use to assess fruit quality. The rapid changes in the skin color of the pawpaw fruit is one of the main reasons why the fruit has remained uncommercialized for several years (Zhang et al., 2017). Various studies have used the CIELAB tristimulus color values (L*, a*, b*) to assess the color of the skin of the pawpaw fruit as well as the color of the pulp. The fruit is known to undergo changes in skin color from yellowish green to brown or black after 3 days of postharvest storage at room temperature (Donno et al., 2014). According to Zhang et al. (2017), pawpaw fruits are highly susceptible to enzymatic discoloration caused by the activity of the polyphenol oxidase (PPO) enzyme. The enzyme catalyzes the conversion of colorless *o*-diphenol in the pulp into *o*-quinones that generate dark melanin after polymerization (Yoruk & Marshall, 2003).

The skin color of the pawpaw fruit is also dependent on the cultivar, although most cultivars have a similar color. Studies have shown that the skin of ripe fruits from the Sunflower cultivar has a skin color with L* value of 70.30, a* value of -10.28, b* value of 38.89, and a hue angle of 99.04º, while the skin of ripe fruits from the Wilson cultivar has

a skin color with L* value of 66.49, a* value of -5.33, b* value of 39.76, and hue angle of 95.93º (Lolletti et al., 2021). Similarly, the color of the ripe fruit pulp varies slightly depending on the cultivar. The pulp of ripe Overleese fruits has L* value of 79.3, a* value of 2.1, b* value of 34.6, and a hue angle of 87º whereas the pulp of ripe NC-1 fruits has L* value of 77.1, a* value of 10.1, b* value of 45.9, and hue angle of 78º (Brannan et al., 2015). Further, Lolletti et al. (2021) found that there were slight differences in skin and pulp color parameters in different crop years, although these differences were not statistically significant except for the b* values. In addition, they found that there are significant interactions between cultivar and crop year for the skin b* and pulp b* values.

2.7 Textural properties of the North American pawpaw fruit

Generally, it has been thought that the skin color of pawpaw fruits is not a reliable indicator of ripeness since different cultivars have different skin color which may not be very different from the skin color of unripe fruits, hence, the firmness (or hardness) of the pawpaw fruit has generally been used to assess ripeness (Adainoo et al., 2023). In a study that assessed the textural properties of mature unripe pawpaw fruits which were harvested and left to ripen in the laboratory in comparison to pawpaw fruits that ripened on the tree before they were harvested, it was found that the hardness of mature unripe fruits is considerably high (9.7–27.0 kg) and reduces to 0.2–0.3 kg within 36 days depending on the cultivar Mcgrath & Karahadian (1994). Further, another study by Archbold & Pomper (2003) found that unripe fruits that were stored at room temperature had a rapid loss of firmness within 15 days and unripe fruits that were stored at 4ºC had a gradual loss of firmness over 40 days. Also, they noted that mature fruits that were ripening had a significantly lower firmness (about 6N) than those unripe fruits (about 50N). From these

findings, the authors concluded that cold storage of pawpaw fruits at 4ºC could delay the loss of textural properties (firmness or hardness) (Archbold & Pomper, 2003).

2.8 Physicochemical properties of the North American pawpaw fruit

There are several physicochemical properties of food, hence, the food material being studied needs to be taken into consideration in selecting the physicochemical properties to best characterize the food material (Igual & Martínez-Monzó, 2022). For fruits, some of the important physicochemical properties include pH, titratable acidity, soluble solids content, and density, among others. Physicochemical properties and changes in physicochemical properties of foods depend on the constituent and the nature of the food material (fluid or solid) (Igual & Martínez-Monzó, 2022). To date, only a few studies have evaluated the physicochemical properties of the North American pawpaw fruit. Some studies have analyzed some of the chemical properties such as the specific acids and sugars in the fruit which may have a bearing on the fruit's physicochemical properties.

According to Nam et al. (2018), the pulp of ripe pawpaw fruits contains malic acid, citric acid, oxalic acid, acetic acid, and formic acid, with acetic acid being the predominant acid in the fruit at a concentration of 61.59±0.92mg/100g of fresh weight. The concentration of the organic acids present in the fruit pulp is significantly lower than the concentration of organic acids present in other fruits like mango that has 0.7g/100g fresh weight of citric acid and 0.5g/100g fresh weight of malic acid, and banana which has 544.30±2.80mg/100g fresh weight of malic acid and 341.67±0.32g/100g fresh weight of citric acid (Lebaka et al., 2021; Maduwanthi & Marapana, 2019). Based on these, it can be said that pawpaw pulp has a relatively higher pH than mango and banana. Galli et al. (2008) found that pawpaw pulp has a pH of 6.54 at harvest, remains relatively stable for 2-6 weeks

of storage and eventually drops to 5.3. Further, findings by Donno et al. (2014) and Brannan (2016) show that ripe fruits of the Davis cultivar have a pH of 4.40±0.29, ripe fruits of the Green River Belle cultivar have a pH of 6.3, fruits of the Susquehanna cultivar have a pH of 5.9 and fruits of the Sunflower cultivar have a pH of 6.2, suggesting that the pH of the fruit is influenced by the cultivar and possibly where the fruits are grown.

Another physicochemical property that is important for evaluating fruit quality is the total soluble solids content. Total soluble solids (TSS) content is a measure of how much soluble solids are present in a sample. Generally, it is used to estimate how much dissolved sugar is present in fruits. Studies have found that the unripe pawpaw fruit has a very low sugar content which increases as the fruit ripens (Park et al., 2022). A study by Mcgrath & Karahadian (1994) found that the total soluble solids content of unripe pawpaw fruit is between 7.4 – 8.6ºBrix while the total soluble solids content of ripe pawpaw fruit is between 19.0 – 25.9ºBrix depending on the cultivar. In addition, analysis of a variety of cultivars found that Lynn's favorite and Susquehanna cultivars have relatively higher (approximately 28ºBrix) total soluble solids contents compared to other cultivars (Brannan, 2016; Brannan et al., 2015).

In addition to the acidity and soluble solids content of the pawpaw fruit, there are some other important properties that may be crucial for monitoring the quality of the fruit and even designing storage systems for extending the shelf life of the fruit. One of such properties is the density of the pulp and fruit. With these, scientists can develop systems to ensure a longer shelf life of the fruit to aid its commercialization.

2.9 Preservation of the pawpaw fruit and pulp

Studies have shown that after 3 days of postharvest storage at room temperature, there is an increased production of flavor volatiles, increase in soluble solids, softening of the flesh, color changes and an increased enzymatic activity (Donno et al., 2014). A few studies have been conducted in an attempt to develop technologies to extend the shelf life of the whole fruit and the fruit pulp. In a study by Galli et al. (2008), whole pawpaw fruits were subjected to extended periods of cold storage at different temperatures to understand how cold storage affects the quality of the fruit over time. They found that mature pawpaw fruits stored at 2-4ºC for 4 weeks ripened normally but those stored at -2ºC did not follow the normal pattern of ripening and those stored at 6ºC were overripe after the storage period. Further, they found that fruits stored at 2-4ºC had a reduced respiration rate, lower ethylene production, lower firmness and decreased pH after 6-8 weeks of storage. The authors concluded that storage of mature pawpaw fruits at 2-4ºC should be limited to 4 weeks because after that storage period, the fruits lose the ability to continue ripening and there are evident signs of chilling injury at colder storage temperatures for longer cold storage periods.

One of the main challenges with pawpaw pulp is the rapid browning it undergoes after exposure to air. Zhang et al. (2017) studied the effect of high-pressure processing, browning treatments and refrigerated storage on the sensory properties and polyphenol oxidase activity in pawpaw pulp. They found that the addition of chemical browning inhibitors (stevia and ascorbic acid) did not have a significant effect on the color of the pulp during storage. Further, they noted that PPO activity and the color of pawpaw pulp were significantly affected by refrigerated storage with PPO activity declining after 24

hours of storage and remaining unchanged for the rest of the 45-day storage period. The study revealed that high-pressure processing can significantly decrease polyphenol oxidase (PPO) activity, but it did not completely inhibit the activity of the enzyme, making high-pressure processing potentially an excellent processing technique to retain the color of the pulp during storage.

In addition, another study by Brannan & Wang (2017) found that the addition of ascorbic acid to pawpaw pulp results in a 69% reduction in PPO activity compared to vacuum-treated pulp. However, the addition of n-acetylcysteine was found to be significantly more effective than ascorbic acid. The addition of n-acetylcysteine almost completely inhibited the activity of PPO in the pawpaw pulp samples, thereby preventing the browning of the pulp during 8 months of frozen storage.

Brief Synopsis: Chapter 3 contains analyses of some physical and physicochemical properties of frozen North American pawpaw fruits from eight different cultivars and their tissues (peels, pulp, and seeds). This chapter also presents the processing potential of the fruits from the different cultivars studied. The content of this chapter has been published in the *Frontiers in Nutrition* journal.

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Chapter 3

Physical characterization of frozen fruits from eight cultivars of the North American pawpaw (Asimina triloba)

Abstract

Pawpaw (*Asimina triloba* [L.] Dunal) is an underutilized fruit native to North America. The fruit has a short shelf life, and browns and softens rapidly after harvesting. These characteristics present a challenge to the advancement of pawpaw as an economically viable specialty crop. This study evaluated the physical characteristics of frozen fruits from eight cultivars of the pawpaw fruit to establish the processing potential of pawpaw fruits. The results show that freeze-thaw cycle may have influenced the peel thickness, peel color, and pulp color of the fruits. Fruits of the Susquehanna cultivar had the highest fruit weight and pulp weight, making them potentially the most suitable for pulp processing. The pawpaw fruits had almost neutral pH ranging between 6.07±0.21 and 6.47±0.11, which could contribute to the rapid browning on exposure to air since an acidic pH is important for slowing enzymatic browning. To aid pawpaw juice extraction, enzymatic treatments may be necessary to increase the juice yield from the pulp. Overleese fruits may be the best for pawpaw juice production. These findings can aid in the selection of processing equipment and guide processors in their efforts to utilize pawpaw fruits to avoid postharvest and post-processing losses.

3.1 Introduction

The North American pawpaw (*Asimina triloba* [L.] Dunal) is a fruit tree that belongs to the same family (Annonaceae) as several widely cultivated tropical fruit trees such as soursop/graviola (*Annona muricata* L.), custard apple (*Annona reticulata* L.) and sugar apple (*Annona squamosa* L.) (Brannan et al., 2015). The pawpaw is one of the few temperate species of this family and is native to the Eastern region of North America. Pawpaw grows best in places that experience hot summers and cold winters (Ames, 2010). Its distribution spans from the west of New York to southwestern Ontario southwards through Michigan, Illinois, Missouri, and further south to eastern Texas and Florida. The fruit is currently being grown in other countries including South Korea, Japan, Italy, China, Israel, Romania, Portugal, Nigeria, and Belgium (Brannan et al., 2015; Nam, Jang, et al., 2018; Ortutu et al., 2015).

Between 1900 and 1960, more than 56 pawpaw cultivars were named, however, with time, some of them were lost as they were no longer cultivated (Peterson, 1990). Presently, there are about 47 known pawpaw cultivars, and these include both wild selections and bred cultivars (Pomper et al., 2009). Pawpaw fruits are mostly asymmetrical, having an oblong-cylindrical shape with some having globular or arched shapes (Szilagyi et al., 2016). Fruits of the various cultivars differ in their rate of growth and ripening, and physical characteristics such as fruit size, color, texture, and percent of seeds (Pomper et al., 2009). Although the flavor of the fruit has commonly been described to be similar to the combination of banana, mango, and pineapple flavors, other flavor descriptors such as apple, melon, and fresh flavors have been used by trained sensory panelists to describe the flavor of specific cultivars of pawpaw fruit (Duffrin & Pomper, 2006; Pomper et al., 2010).

The fruit's unique flavor and aroma make it suitable for potential applications in baby foods, fruit drinks, ice cream, and as a substitute for bananas in various foods (Bratsch, 2009). The fruit contains 79.14% moisture, 0.38% ash, 1.51% protein, 0.36% lipid, 2.47% crude fiber, 18.61% carbohydrates, and 3.03% dietary fiber (Nam, Jang, et al., 2018). Further, it is known to be a good source of β-carotene, polyphenols, antioxidants, and other important compounds (Lolletti et al., 2021; Nam, Jang, et al., 2018).

Pawpaw fruits can weigh up to 1 kg (Brannan et al., 2015; Brindza et al., 2019). Some of the cultivars that yield large fruits include Convis, IXL, Lynn's Favorite, Overleese, SAA-Overleese, Shenandoah, and Susquehanna, whereas those that yield small fruits are LA Native, Middletown, Rappahannock, and Wilson (Pomper et al., 2009).

It has been observed that a change in the intensity of the peel's green color is not a good measure of the ripeness of the pawpaw fruit because this color change is not consistent for all the genotypes (Archbold & Pomper, 2003). However, the peels change color from green to yellow to brownish black as ripening progresses and the pulp color of a ripe pawpaw fruit ranges from creamy white to yellow to orange (Brannan et al., 2015). Additionally, the pulp browns when exposed to air. This color change is caused by the action of polyphenol oxidases in the fruit pulp (Brannan & Wang, 2017). The fruit contains two rows of black seeds that are about the size of almonds.

Pawpaw fruits have a short shelf life. As pawpaw fruits ripen, the soluble solids concentration increases, however, this is not a good indicator of ripeness (Brannan et al., 2015). The fruits soften within 3 days after harvesting due to their high ethylene production and climacteric respiration (Archbold & Pomper, 2003). By day 5 after harvesting (without refrigeration), the fruit often becomes overripe and too soft to handle (Galli et al., 2008).

These factors coupled with the rapid color changes that occur in the peel and pulp make processing the pawpaw fruit a challenge.

This study aimed to assess the physical characteristics of the frozen pawpaw fruits from eight different cultivars to establish a basis for their processing potential. Understanding the physical characteristics of the pawpaw fruit is important for the selection of advanced cultivars, as well as the design of appropriate processing equipment, to allow for the industrial processing of the fruit and ensure there are no significant losses during processing operations.

3.2 Materials and Methods

3.2.1 Pawpaw and Mango Samples

Fifty-three (53) pawpaw fruits from eight cultivars (10-35, PA-Golden, Shenandoah, Sunflower, Susquehanna, Wells, Overleese, and Wilson) were harvested from two orchards (designated Lower and Upper orchards; 3 km apart) at the Southwest Research Center of the University of Missouri, Mt. Vernon, MO (lat. 37.08582, long. -93.86713, and lat. 37.07146, long. -93.87870 respectively). The Lower orchard had a fertile alluvial soil that was deep and well-drained, whereas the Upper orchard had a less fertile soil with a shallow fragipan that required more irrigation than the Lower orchard. The trees generally grew larger and more vigorously in the Lower Orchard. The fruits were harvested at peak ripeness (determined by the pitting on the skin when the fruit is gently pressed with a finger) in Sept./Oct. 2020. The fruits from the respective cultivars were separated by placing them in separate zippered plastic storage bags and stored whole in a deep freezer

(-18ºC) immediately after harvesting for 14 days prior to transportation to the laboratory for analyses. Fruits were thawed in tepid water at ~25ºC for approximately 10 minutes before analyses. Fresh mango (Kent variety) samples were purchased from Walmart in Columbia, MO and evaluated to provide a basis for comparison.

3.2.2 Fruit and fruit component weight and size

The total weight, seed weight, and peel weight for each fruit were measured using an analytical balance. Pulp weight was obtained by difference. The number of seeds per fruit were recorded, except for the mango fruits, which only have one seed. Peel thickness was measured with a Vernier caliper (Akbarpour et al., 2009). The fruit length, width, and thickness were also measured with a Vernier caliper as demonstrated in Figures 3-1A and 3-1B. All measurements were done in five replicates.

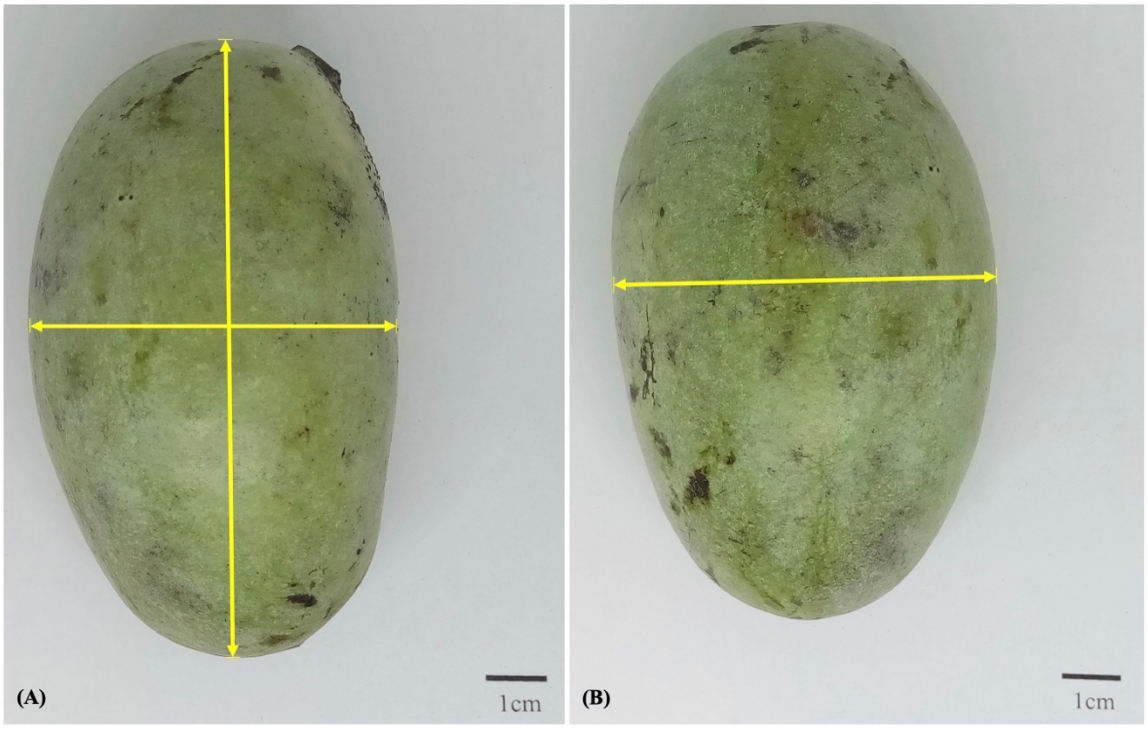

Figure 3-1: Pictorial demonstration of how (A) fruit length and width and (B) thickness were measured.

3.2.3 Fruit color

Fruit peel color, outside pulp color (the pulp just beneath the peel), internal pulp color, and seed color were measured according to the method described by Nambi et al. (2015) using the Hunter LAB color meter (Chroma Meter CR-410, Konica Minolta). All color readings were done in five replicates at five different points on the peel, outside pulp, internal pulp, and seeds. The L*, a*, and b* readings were recorded where, L* is the degree of lightness to darkness, a* is the degree of redness to greenness, and b* is the degree of yellowness to blueness.

3.2.4 Fruit shape index

Fruit shape index (FSI) was measured as the ratio of the maximum fruit length to the maximum fruit width as described by Brewer et al. (2006).

$$\text{Fruit shape index} = \frac{\text{Maximum fruit length}}{\text{Maximum fruit width}}$$

3.2.5 Fruit volume

Fruit volume was measured by the displacement method using a graduated measuring cylinder (Ngouajio et al., 2003). The measuring cylinder was filled with water to a specific volume and the change in displacement of water after gently dropping fruit into the water was recorded as the volume of the fruit in cm^3. The measurements were taken in five replicates for each fruit.

3.2.6 Pulp density

The density of pawpaw and mango pulps was determined according to the procedure described by Bon et al. (2010) with some modifications. A pycnometer (Ultrapycnometer 1000, Quantachrome Instruments) and an analytical balance were used to determine the pulp density in triplicates at 25ºC. 5g of pulp was first weighed into the small pycnometer cell. The pycnometer was set to take five density readings and take averages of the five readings. This was done in triplicates.

3.2.7 Determination of juice content

The juice content was determined according to the methods described by Agbaje et al. (2020) and Jamil et al. (2015). Fruits were washed with tap water followed by distilled water to remove foreign materials from the fruit. The fruit was hand peeled and the pulp

separated from the peels and seeds, then blended to reduce size and aid juice extraction. The juice in a known weight of the blended fruit pulp was extracted using a clean white muslin cloth. The juice content was calculated as a percentage of the weight of the fruit. Juice contents were determined in triplicates.

$$\text{Juice content} = \frac{\text{Weight of extracted juice}}{\text{Weight of blended fruit}} \times 100$$

3.2.8 Determination of pH and titratable acidity

The pH of the fruit was measured using a digital pH meter (SevenCompact S220, Mettler Toledo) at room temperature (25ºC). The measurements were taken in five replicates. Titratable acidity was determined according to the AOAC Official Method 942.15 (AOAC, 2000a). Since the pulp of the fruits was quite dry at the time of the titratable acidity experiment, 5g of the fruit pulp was mixed with 25g of distilled water, blended in a kitchen blender for 2 minutes to obtain a homogeneous mixture, and titrated against 0.1N NaOH using phenolphthalein as indicator. The analyses were performed in triplicates and reported as acetic acid equivalents since the predominant acid in pawpaw is acetic acid (Nam, Jang, et al., 2018).

$$\text{Titratable acidity} = \frac{\text{NaOH normality} \times \text{Titre value} \times \text{Acetic acid eq. weight} \times 100}{\text{Sample weight} \times 1000}$$

$$\text{Acetic acid eq. weight} = 60.052 \text{ g}$$

3.2.9 Determination of total soluble solids

Total soluble solids (TSS) content was measured according to the AOAC Official Method 932.14C (AOAC, 2000a) using a digital refractometer (HI96800, Hanna

Instruments) at room temperature (~25ºC). 5g of the fruit pulp was mixed with 25 ml of distilled water and blended in a kitchen blender for 2 minutes to obtain a homogeneous mixture. The readings were multiplied by the dilution factor (5). The total soluble solids measurements were taken in triplicates and recorded as ºBrix.

3.2.10 Determination of thermophysical properties

The thermophysical properties of pawpaw pulp (only Sunflower cultivar) was determined by differential scanning calorimetry (DSC) as described by Gundurao et al. (2011) with some modifications. The differential scanning calorimeter (TA Q20, TA Instruments) calibrated with indium for heat flow and the temperature was equipped with a cooling system that monitored temperatures down to -90ºC. Nitrogen gas was used as a purge gas with a flow rate of 50ml/min. About 14 mg of pawpaw pulp was weighed into aluminum pans which were hermetically sealed to avoid moisture loss. An empty sealed aluminum pan was used as a reference. To determine the glass transition temperature and the change in specific heat capacity, sealed pans with pawpaw pulp samples were cooled to -30ºC and subjected to a programmed heating rate of 10ºC/min to 200ºC. The DSC data were analyzed with the Universal Analysis Software (version 4.5A) for thermal analysis.

3.2.11 Microstructure of pawpaw pulp (Scanning electron microscopy)

Pulp samples from near the seeds and pulp samples further from the seeds were collected from Susquehanna pawpaw fruits and processed for scanning electron microscopy (SEM). Unless otherwise stated, all reagents were purchased from Electron Microscopy Sciences and all specimen preparation was performed at the Electron Microscopy Core Facility, University of Missouri. Tissues were fixed in 2%

paraformaldehyde, and 2% glutaraldehyde in 100 mM sodium cacodylate buffer pH=7.35. Next, fixed tissues were rinsed with 100mM sodium cacodylate buffer, pH 7.35 containing 130mM sucrose. Secondary fixation was performed using 1% osmium tetroxide (Ted Pella, Inc., California, USA) in cacodylate buffer using a Pelco Biowave (Ted Pella, Inc., California, USA) operated at 100Watts for 1 minute. Specimens were next incubated at 4^{o}C for 1 hour, then rinsed with cacodylate buffer and further with distilled water. Using the Pelco Biowave, a graded dehydration series (per exchange, 100 Watts for 40s) was performed using ethanol. Samples were dried using the Tousimis Autosamdri 815 (Tousimis, Maryland, USA) and samples were sputter coated with 20nm of platinum using the EMS 150T-ES. Sputter Coater Images were acquired with a FEI Quanta 600F scanning electron microscope (FEI, Oregon, USA) at a voltage of 5.00kV and magnifications of 100x, 500x, and 1000x.

3.2.12 Statistical Analysis

All experimental data are presented as mean values ± SD (standard deviation). The data were analyzed by analysis of variance (ANOVA) and Tukey's test ($p<0.05$) for significant differences using JMP 14.0.0 software (SAS Institute Inc., Cary, USA). Microsoft Excel version 16.46 was used for further processing of the data into tables and graphs.

3.3 Results

3.3.1 Size and morphological characteristics of pawpaw

The fruit size data shows that though the Lower orchard had better soil conditions than the Upper orchard, the differences in the soil, and environmental conditions in which

pawpaw fruits are grown, affect the fruit size of the cultivars differently. Fruits of the 10-35, PA-Golden, Shenandoah, and Wells cultivars from the Upper orchard had slightly higher average lengths than fruits of those cultivars in the Lower orchard. However, there were no statistical differences between fruits of these cultivars from the two orchards at $p<0.05$ as shown in Figure 3-2A. Meanwhile, fruits of the Sunflower and Susquehanna cultivars in the Lower orchard were longer than the fruits of these cultivars in the Upper orchard with statistically significant differences at $p<0.05$. Further, apart from the statistical differences between the widths of the 10-35 fruits in the Lower orchard and the Upper orchard, there were no significant differences between the widths of the fruits from the two orchards for all the other cultivars except for the Susquehanna fruits from the Lower orchard which had a significantly higher fruit width (Figure 3-2B).

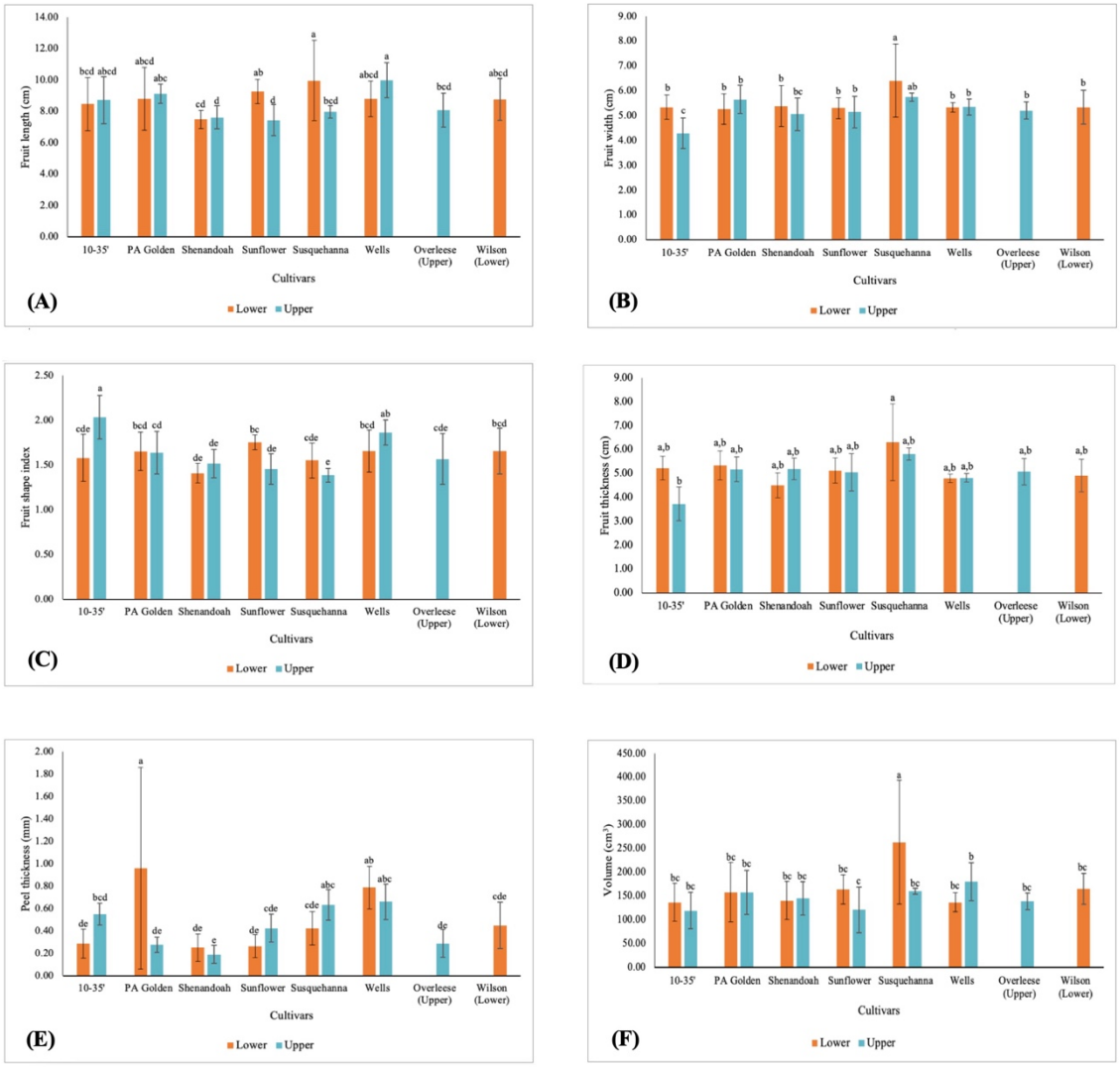

Figure 3-2: Dimensional characteristics of pawpaw fruits from different cultivars in the Lower and Upper orchards showing (A) fruit length, (B) fruit width, (C) fruit shape index, (D) fruit thickness, (E) peel thickness and (F) volume of fruits.

From the fruit shape index (FSI) data (Table 3-1 & Figure 3-2C), fruits of the 10-35 cultivar in the Upper orchard had significantly higher FSI (2.04±0.24) than those in the Lower orchard and all the other cultivars. The differences in the fruit thickness among the cultivars were statistically insignificant at $p<0.05$ (Figure 3-2D). From the data obtained, Susquehanna fruits recorded the highest average volume (217±110 cm^3), which was also significantly different from the fruits from the other cultivars at $p<0.0001$ (Table 3-1). Further, among the fruits from the two orchards, Wells and Sunflower fruits harvested from the Upper orchard and the Susquehanna fruits from the Lower orchard showed significant differences in volume. The volume of the fruits from the other cultivars were not statistically different at $p<0.05$ (Figure 3-2F).

Table 3-1: Morphological characteristics of pawpaw fruits from different cultivars grown in southwest Missouri, 2020.

Cultivar	Volume (cm^3)	Fruit length (cm)	Fruit width (cm)	Fruit thickness (cm)	Fruit shape index	Peel thickness (mm)	Fruit weight (g)	Pulp weight (g)	Peel weight (g)	Seed weight (g)	Number of seeds
10-35	130 ± 40^{b}	8.56 ± 1.62^{abc}	4.95 ± 0.74^{c}	4.64 ± 0.94^{a}	1.75 ± 0.34^{a}	0.38 ± 0.18^{bcd}	137 ± 44^{b}	122 ± 37^{b}	10.1 ± 4.5^{d}	6.4 ± 2.8^{d}	4-8
Overleese	139 ± 17^{b}	8.07 ± 1.09^{bc}	5.20 ± 0.35^{bc}	5.08 ± 0.56^{a}	1.57 ± 0.28^{ab}	0.29 ± 0.12^{cd}	143 ± 14^{b}	109 ± 10^{b}	24.9 ± 2.9^{ab}	8.6 ± 1.7^{bcd}	4-13
PA Golden	158 ± 54^{b}	8.99 ± 1.40^{ab}	5.48 ± 0.62^{b}	5.25 ± 0.56^{a}	1.64 ± 0.23^{ab}	0.57 ± 0.68^{ab}	171 ± 50^{b}	143 ± 39^{b}	17.1 ± 14.7^{bcd}	10.5 ± 2.8^{bc}	3-12
Shenandoah	143 ± 37^{b}	7.58 ± 0.75^{c}	5.16 ± 0.66^{bc}	4.96 ± 0.60^{a}	1.48 ± 0.15^{b}	0.21 ± 0.10^{d}	143 ± 38^{b}	117 ± 35^{b}	16.7 ± 2.3^{bcd}	9.2 ± 2.5^{bcd}	4-8
Sunflower	142 ± 46^{b}	8.36 ± 1.27^{bc}	5.23 ± 0.54^{bc}	5.09 ± 0.67^{a}	1.60 ± 0.20^{ab}	0.34 ± 0.14^{cd}	148 ± 47^{b}	123 ± 39^{b}	14.6 ± 7.8^{cd}	11.1 ± 5.2^{b}	4-12
Susquehanna	217 ± 110^{a}	9.07 ± 2.16^{ab}	6.11 ± 1.15^{a}	6.09 ± 1.23^{a}	1.48 ± 0.17^{b}	0.51 ± 0.18^{abc}	241 ± 135^{a}	208 ± 118^{a}	24.7 ± 16.0^{a}	8.2 ± 4.7^{cd}	3-13
Wells	160 ± 39^{b}	9.41 ± 1.27^{a}	5.34 ± 0.27^{bc}	4.81 ± 0.18^{a}	1.76 ± 0.22^{a}	0.72 ± 0.18^{a}	160 ± 37^{b}	125 ± 25^{b}	18.0 ± 8.9^{abc}	17.2 ± 3.9^{a}	5-13
Wilson	165 ± 33^{b}	8.77 ± 1.33^{abc}	5.34 ± 0.68^{bc}	4.92 ± 0.68^{a}	1.66 ± 0.26^{ab}	0.45 ± 0.21^{bcd}	167 ± 29^{b}	138 ± 22^{b}	12.1 ± 5.2^{cd}	16.6 ± 2.5^{a}	8-10
Control (Mango Fruit)	324±3	9.85±0.18	7.89±0.19	7.20±0.20	1.25±0.05	2.76±0.56	334±20	201±20	97.1±14.2	35.4±3.7	-

Values with the same superscripts are statistically similar at $p<0.05$

Fruit shape index is the ratio of the maximum fruit length to the maximum fruit width

The environmental and soil differences between the Lower and Upper orchards did not affect the weights of the fruits except for the Susquehanna fruits from the Lower orchard, which had significantly heavier fruits (299±158 g) compared to the other cultivars studied (Figure 3-3A). There was no statistical difference ($p<0.05$) in the weights of the fruits among the other cultivars studied (Table 3-1 & Figure 3-3A). Also, the number of seeds per fruit varied widely among the cultivars. Some PA Golden and Susquehanna fruits had as few as three seeds per fruit, whilst some Overleese, Susquehanna, and Wells fruits had as many as 13 seeds per fruit. This contributed to the wide variations in the seed weights as shown in Figure 3-3B. On average, seeds in the Lower orchard 10-35, Shenandoah, Sunflower, and Susquehanna fruits weighed more than seeds of the same cultivars in the Upper orchard (Figure 3-3B). Similarly, there were wide variations in the peel weights (Table 3-1 & Figure 3-3C).

Some of the pawpaw fruits studied had thin peels while others had thick peels because layers of the outside pulp firmly adhered to the peels. However, this was not consistent for all the fruits, which explains why the peel thickness of the Susquehanna fruits from the Lower orchard was significantly lower than that of the PA-Golden fruits from the Lower orchard (Figure 3-2E) but the peel weights of both Susquehanna and PA-Golden fruits from the Lower orchard were not significantly different (Figure 3-3C).

Susquehanna fruits recorded the highest pulp weight (208±118 g). The data (Table 3-1 & Figure 3-3D) show that there were no significant differences ($p<0.05$) in the pulp weights of the other cultivars tested, although PA-Golden and Wilson fruits had slightly more pulp than the others. Moreover, the better soil conditions of the Lower orchard favored the pulp

weight of the Susquehanna, 10-35, and Sunflower cultivars, but not the PA Golden, Shenandoah, and Wells cultivars.

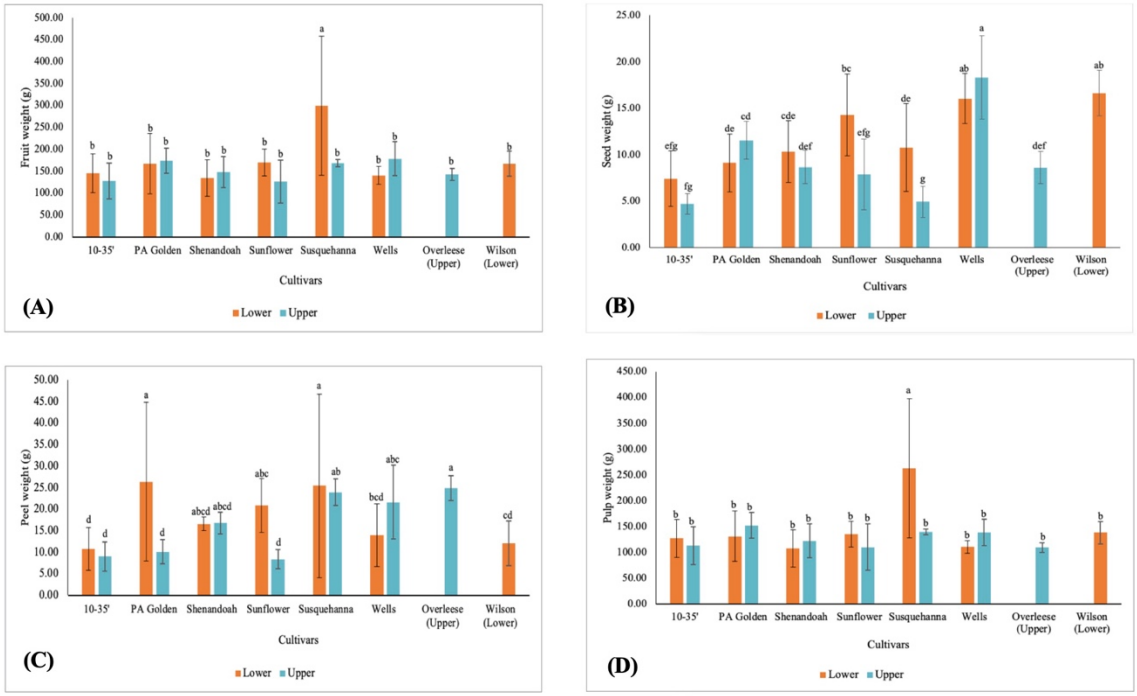

Figure 3-3: Weight of (A) fruits, (B) seeds, (C) peels, and (D) pulp of pawpaw fruits from different cultivars in the Lower and Upper orchards.

3.3.2 Pawpaw color

There were statistical differences in peel color among fruits of the same cultivar from the different orchards ($p<0.0001$) and among fruits of different cultivars ($p<0.0001$). The peels of the Sunflower cultivar fruits were the lightest (43.9±3.6 L*) and had the highest yellowness (24.6±6.2 b*) among the cultivars studied, whereas the peels of the Wells cultivar fruits were the darkest (34.6±5.1 L*) of the cultivars studied (Table 3-2).

The color of the pulp layer just beneath the peels (outer pulp) was measured separately from the color of the pulp because the outer pulp was observed to brown more rapidly than the (inner) pulp. This was observed in the lightness values obtained; the outside pulp lightness values (Table 3-1) for all the cultivars were lower than the lightness values of the pulp indicating the outer pulp was darker than the (inner) pulp (Table 3-1). Further, the results show that the outside pulp lightness for fruits of the Shenandoah, Sunflower, Wilson, PA Golden, Overleese, and Susquehanna cultivars were not statistically different ($p<0.05$) (Table 3-2). However, the lightness of these cultivars was significantly different from the lightness of the outside pulp of fruits of the 10-35 and Wells cultivars ($p<0.0001$). Also, from the data obtained, the pulp of all the pawpaw cultivars studied recorded higher yellowness and lower redness (Table 3-2) whereas the outside pulp recorded lower yellowness and higher redness.

The seeds in the Wilson cultivar fruits were the darkest with an average L* value of 35.3±3.2, whereas seeds in the 10-35 cultivar fruits had a relatively lighter color with an average L value of 45.8±7.3 (Table 3-2).

Table 3-2: Color of the peel, outside pulp, pulp, and seeds of pawpaw fruits from different cultivars grown in southwest Missouri, 2020

	Peel Color			Outside Pulp Color			Pulp Color			Seed Color		
Cultivar	L*	a*	b*	L*	a*	b*	L*	a*	b*	L*	a*	b*
10-35	39.9±4.0^{ab}	3.3±2.9^{bcd}	24.1±4.4^{a}	44.3±6.8^{b}	13.2±3.6^{bc}	33.3±8.2^{cd}	63.2±6.1^{cd}	7.8±4.7^{cd}	39.1±4.9^{d}	45.8±7.3^{a}	1.7±0.8^{d}	-0.6±1.5^{b}
Overleese	39.3±3.5^{abc}	1.5±4.9^{d}	19.1±3.2^{bc}	50.8±3.9^{a}	10.3±2.0^{c}	35.7±7.2^{bc}	67.9±2.9^{bc}	9.4±1.8^{bc}	44.7±1.9^{abc}	41.0±8.6^{abc}	1.6±0.5^{d}	-1.0±0.9^{b}
PA Golden	38.1±2.7^{bc}	2.5±3.7^{cd}	16.2±3.8^{cd}	51.1±7.3^{a}	10.7±3.6^{c}	34.0±5.3^{bcd}	64.3±8.3^{c}	8.9±2.0^{bc}	30.3±11.9^{e}	38.8±7.2^{bcd}	2.8±1.0^{ab}	5.2±9.9^{a}
Shenandoah	37.9±3.3^{bc}	8.4±3.0^{a}	20.0±4.9^{bc}	53.2±4.3^{a}	10.4±1.5^{c}	38.0±4.0^{abc}	72.1±1.9^{ab}	7.6±1.6^{cd}	45.5±3.2^{a}	43.0±3.9^{ab}	2.7±0.9^{abc}	1.1±2.4^{b}
Sunflower	43.9±3.6^{a}	5.6±7.5^{abc}	24.6±6.2^{a}	53.2±4.5^{a}	15.5±4.3^{b}	42.6±7.1^{a}	74.3±3.2^{a}	6.9±2.0^{d}	44.8±3.8^{ab}	39.6±6.7^{bcd}	2.0±0.7^{cd}	-0.1±1.5^{b}
Susquehanna	39.8±12.0^{b}	5.8±3.5^{ab}	13.0±7.2^{d}	50.4±7.6^{a}	20.4±6.0^{a}	39.4±12.5^{ab}	62.7±6.5^{cd}	13.8±2.3^{a}	40.2±6.5^{bcd}	42.4±5.4^{ab}	2.2±1.9^{bcd}	0.5±4.5^{b}
Wells	34.6±5.1^{c}	3.6±3.7^{bcd}	14.3±5.4^{d}	43.9±5.3^{b}	11.8±4.4^{c}	28.7±5.1^{d}	59.3±8.9^{d}	10.1±2.1^{b}	33.1±7.3^{e}	35.7±3.7^{d}	1.9±0.5^{d}	0.7±1.7^{b}
Wilson	41.8±3.9^{ab}	6.6±3.8^{ab}	22.4±5.2^{ab}	52.4±4.2^{a}	9.3±3.1^{c}	36.9±6.1^{abcd}	69.9±2.2^{ab}	9.3±2.6^{bc}	38.7±2.9^{cd}	35.3±3.2^{cd}	3.3±1.1^{a}	2.8±2.1^{ab}
Control (Mango Fruit)	53.5±6.8	9.7±10.8	35.2±9.0	75.2±2.3	10.3±1.9	71.0±3.6	70.7±2.6	13.3±2.7	71.2±2.9	70.5±2.3	6.2±1.5	51.5±3.9

L*: high values indicate light colored, low values indicate dark colored; a*: high values indicate redness, low values indicate greenness; b*: high values indicate yellowness, low values indicate blueness

Values with the same superscripts are statistically similar at $p<0.05$

3.3.3 Physicochemical and thermal properties of pawpaw pulp

The pH of the fruits from all the cultivars and sites ranged between 6.07±0.21 and 6.47±0.11 (Figure 3-4A). Overleese fruits recorded the highest pH (6.42±0.17), and the Susquehanna and Sunflower fruits both from the Upper orchard had the lowest pH (6.07±0.21 and 6.07±0.18 respectively) (Table 3-3 and Figure 3-4A). While the titratable acidity of the pawpaw cultivars was statistically similar at $p<0.05$ (Figure 3-4B), the pH values for the fruits among the different cultivars and the sites were statistically different ($p<0.0001$).

The juice content of the pawpaw fruits varied with statistical significance at $p<0.0001$. The juice content of the fruits ranged between 47.7±21.8% and 74.2±5.1% for fruits of different cultivars and sites (Figure 3-4C). On average, Overleese fruits recorded the highest juice content (66.8±7.0%). The same method was used to determine the juice content in fresh mango fruits in this study and it was found that the juice content in the pawpaw cultivars was lower than the juice content in mangoes (73.8±3.2%) as shown in Table 3-3.

The pulp density of the cultivars studied ranged between 1.06±0.10 g/cm^3 and 1.19±0.03 g/cm^3 (Figure 3-4D). However, there were no significant differences among the cultivars and the sites. Also, the pulp density of the pawpaw fruits was similar to the pulp density of the mango fruits examined (1.15±0.04 g/cm^3).

The data obtained show that Susquehanna fruits from both orchards recorded the highest TSS concentration (Figure 3-4E) with an average of 14.38±1.16 ºBrix (Table 3-3).

PA Golden fruits had the lowest TSS. The 10-35, Shenandoah and Wilson cultivar fruits had similar TSS concentrations (Table 3-3).

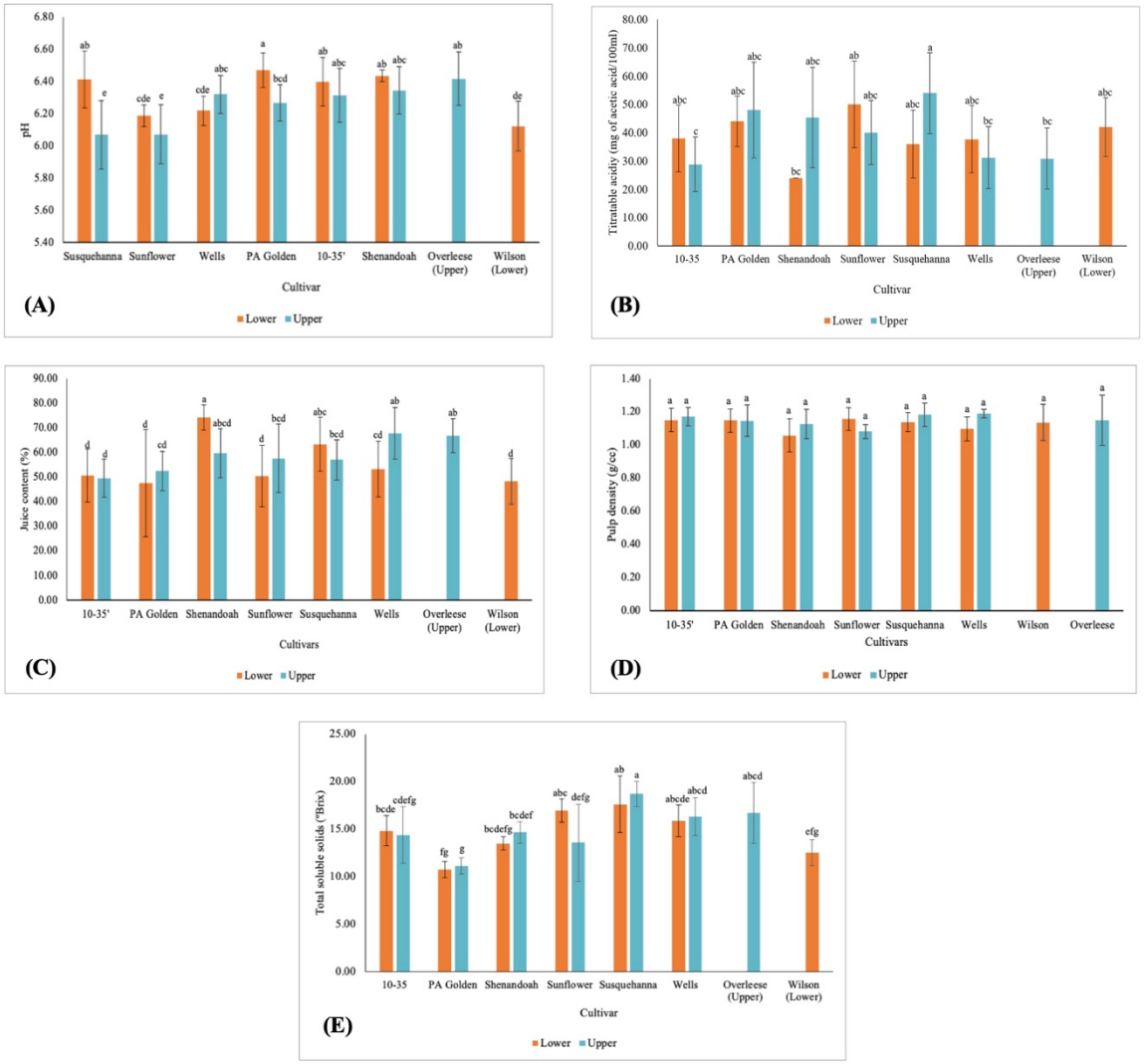

Figure 3-4: Physicochemical properties of pawpaw fruits from different cultivars in the Lower and Upper orchards showing (A) pH, (B) titratable acidity, (C) juice content, (D) pulp density, and (E) total soluble solids concentration.

Table 3-3: Physicochemical properties of pawpaw fruits from different cultivars grown in southwest Missouri, 2020

Cultivar	Pulp density (g/cm^3)	Juice content (%)	pH	Total soluble solids (ºBrix)	Titratable acidity (mg of acetic acid/100ml)
10-35	1.16 ± 0.06^{a}	50.1 ± 9.4^{c}	6.36 ± 0.16^{abc}	14.64 ± 2.32^{bc}	33.82 ± 11.80^{a}
Overleese	1.15 ± 0.15^{a}	66.8 ± 7.0^{a}	6.42 ± 0.17^{a}	16.71 ± 3.17^{ab}	30.86 ± 10.84^{a}
PA Golden	1.15 ± 0.09^{a}	50.4 ± 15.7^{c}	6.35 ± 0.15^{abc}	11.00 ± 0.87^{d}	46.67 ± 14.91^{a}
Shenandoah	1.10 ± 0.10^{a}	64.5 ± 11.0^{a}	6.38 ± 0.13^{ab}	14.38 ± 1.16^{bc}	40.00 ± 17.89^{a}
Sunflower	1.12 ± 0.07^{a}	54.0 ± 13.7^{bc}	6.13 ± 0.15^{d}	15.27 ± 3.45^{b}	45.00 ± 14.39^{a}
Susquehanna	1.16 ± 0.07^{a}	60.2 ± 10.2^{ab}	6.24 ± 0.26^{cd}	18.17 ± 2.38^{a}	45.00 ± 15.97^{a}
Wells	1.14 ± 0.07^{a}	60.9 ± 13.0^{ab}	6.27 ± 0.12^{bc}	16.14 ± 1.88^{ab}	33.88 ± 11.81^{a}
Wilson	1.14 ± 0.11^{a}	48.2 ± 9.3^{c}	6.12 ± 0.15^{d}	12.54 ± 1.36^{cd}	42.00 ± 10.39^{a}
Control (Mango Fruit)	1.15±0.04	73.8±3.2	4.40±0.29	12.78±1.40	255.11±113.89*

Values with the same superscripts are statistically similar at $p<0.05$.

*Titratable acidity of mango was calculated as milligrams of citric acid/100ml

The DSC data obtained show that the glass transition of pawpaw pulp occurs at -8.87ºC accompanied by a change in specific heat capacity of 4.404 kJ kg^{-1} K^{-1} (Figure 3-5A). The peak temperature of ice melting in frozen pawpaw pulp occurs at -0.86ºC and the thermal decomposition of the pulp occurred at 113.42ºC (Figure 3-5B).

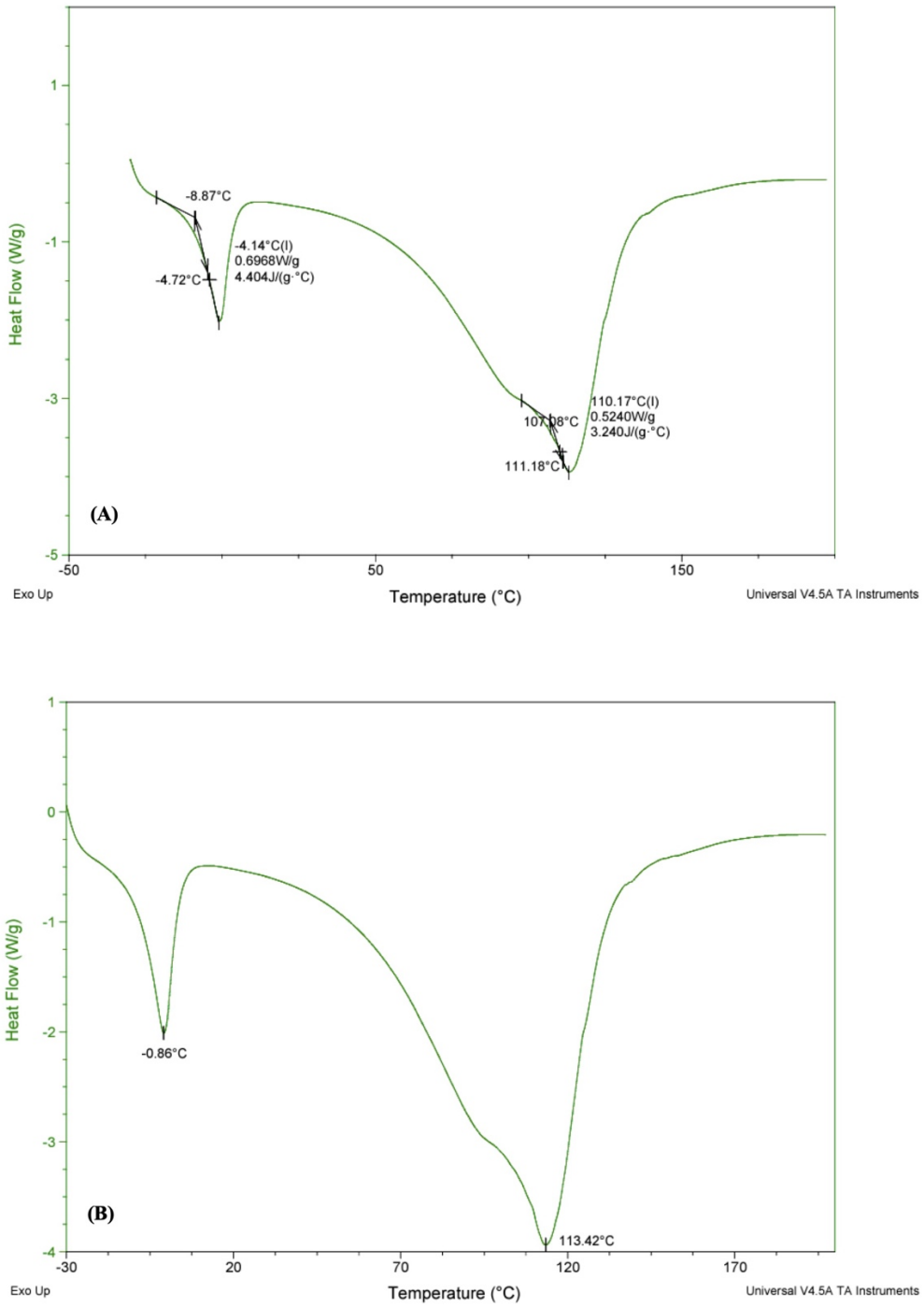

Figure 3-5: DSC thermogram of pawpaw pulp showing (A) glass transition temperature and specific heat capacity, and (B) melting and thermal degradation temperatures.

3.3.4 Microstructure of pawpaw pulp

The SEM (scanning electron microscope) images show a clear distinction in the microstructure of the pulp close to the seed and the pulp further from the seeds (Figure 3-6). The pulp closer to the seeds showed a smoother surface with no fibers (Figures 3-6A-C), whereas the pulp further from the seeds showed a more irregular surface with fibers (Figures 3-6D-E).

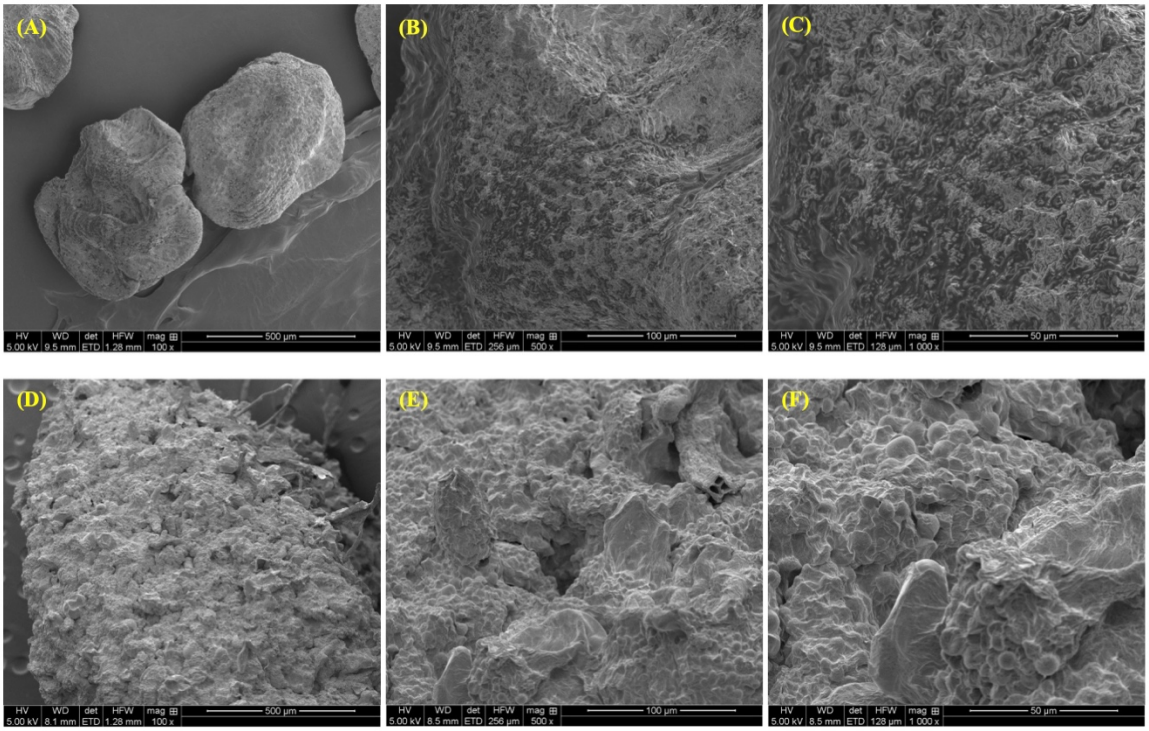

Figure 3-6: Scanning electron microscope images showing pawpaw pulp close to the seeds (A–C) and pawpaw pulp further from the seeds (D–F) at magnifications of 100x, 500x, and 1000x.

3.4 Discussion

3.4.1 Size and morphological characteristics of pawpaw

Fruit size is an important characteristic that is needed in the selection and design of appropriate processing equipment and is also important in cultivar development. Factors that are known to influence fruit size include genetics, crop load on trees, tree age and vigor, soil nutrients, water supply, pollination, and environmental factors like temperature, humidity, pests, and disease. For pawpaw, studies show that fruit size is affected by cultivar (Francino, 2019), and this was observed in the variations in the fruit lengths and widths. The length and width of the fruits studied were all within the range reported for fresh fruits by (Donno et al., 2014). This indicates that the fruit length and width were not affected by the freeze-thaw cycle. FSI is an indicator of fruit shape influenced by the genetic makeup of the fruit. FSI greater than 1 indicates an elongated fruit, FSI equal to 1 indicates a round fruit, and FSI less than 1 indicates a squat fruit (Brewer et al., 2006). The FSI data show that all the fruits analyzed were elongated, but the fruits of the 10-35 cultivar were the most elongated.

Weight is often used as a quality indicator for fruits and many other agricultural products. Generally, fruits that weigh more have a higher pulp weight, which results in more efficient processing. However, it is important to consider other characteristics of the pulp aside its weight (such as the pH, titratable acidity, total soluble solids content among others) to achieve a desirable quality product when processing the fruit. Hence, if processors choose Susquehanna fruits based on their high pulp weight per fruit, it would also be necessary to carefully consider how the properties of the Susquehanna pulp could

influence the quality characteristics of the product they intend to make from the fruit. The weights of the Susquehanna, Wells, and Wilson, fruits were higher than the average weights for the same cultivars as reported by (Pomper et al., 2009). On the other hand, the weight of the Overleese fruits was lower than the average reported by Pomper et al. (2009), but Sunflower fruits had similar weights compared to the average reported by Lolletti et al. (2021). It is unclear if freezing had any effect on the weights of the fruits studied. The differences in the experimental data and reported data may have resulted from the differences in the soil quality and environmental conditions of the Lower and Upper orchards as compared to the sites from which the fruits for reported data were obtained. Fruit volume is an important quality index that is used to predict the best time to harvest fruits (Hahn & Sanchez, 2000) and to determine fruit expansion rate (Ngouajio et al., 2003). The volume of the fruits followed a similar trend as the weight of the fruits; the heavier fruits had high volumes.

Peel thickness provides an understanding of how easily fresh fruits may bruise during handling and transportation (Mohammad Shafie et al., 2015). Additionally, the peel thickness can provide some guidance in the selection and/or design of appropriate industrial peelers to allow for efficient peeling of the fruit before pulp extraction and processing. Peel thickness is influenced by the maturity of fruits; peels of more matured fruits are thinner compared to peels of less matured fruits. Further, peel thickness is an important parameter associated with fruit quality (Bizzani et al., 2017) and because the fruits used in this study were frozen and thawed prior to analyses, it is likely that the peel thickness of the fruits were affected by the freeze-thaw cycle prior to measurements. Ripe pawpaw fruits are delicate and easily damaged, hence breeding or producing fruits with

thicker peels should significantly reduce bruising and losses that may occur during post-harvest transportation and handling. Studies have shown that fruits with thicker peels are less susceptible to bruising as observed in fruits like pomegranates (Hussein et al., 2019; Mohammad Shafie et al., 2015) and banana (Bugaud et al., 2014). Hence, for fresh pawpaw marketing, fruits of the Wells cultivar may be preferred as they may not bruise as easily during handling compared to fruits of the other cultivars. Generally, in industrial fruit pulp extraction and processing, various peeling technologies are used. These peeling technologies include mechanical peelers which may be calibrated to peel fruits with peel thickness ranging between 1mm and 4mm (Chahal & Singh, 2021; Mahawar et al., 2020). However, since the pawpaw fruits have thinner peels (4-13 times thinner than those of mangoes), industrial peelers for other fruits of similar shape and size (like mangoes) may be recalibrated for peeling of pawpaw fruits during industrial processing of pawpaw fruits.

3.4.2 Pawpaw color

Unlike other fruits where the peel color can be used to determine ripeness, peel color alone is not a good indicator of ripeness in pawpaw fruits (Archbold & Pomper, 2003). Browning of the peel and pulp results in lower lightness (L*) values (Subhashree et al., 2017), hence, the lightness and darkness of the pawpaw fruit peels could have been a result of the degree of browning that might have occurred in the peels possibly due to the chill injury that had occurred in the peels of the fruits during the freezing of the fruits. The fruits of the Wells cultivar have peels that had the darkest peel color compared to the fruits of the other cultivars. Also, the Sunflower and Wilson fruits peel studied were darker, redder, and less yellow than the Sunflower and Wilson fruit peels studied by Lolletti et al. (2021),

confirming the effect of freezing on the peel color of the fruits. The lightness of the peels of the fruits was quite consistent for fruits of the same cultivar from the different orchards, indicating that the differences in soil and environmental conditions did not have much effect on the fruit colors even though the freeze-thaw cycle could have affected the data obtained. Hence, to get a better understanding of the effect of soil and environmental conditions on pawpaw fruits, further studies with fresh fruits would need to be conducted.

The data obtained shows that the outer pulp layer had a higher degree of redness compared to the pulp which may have resulted from a higher polyphenol oxidase (PPO) activity in the outer pulp layer. A high PPO activity results in more browning (Queiroz et al., 2008). Based on this, during the processing of the fruits, high-pressure processing can be employed to effectively inhibit the activity of PPO in pawpaw pulp without affecting the sensory attributes of the pulp (Zhang et al., 2017). Alternatively, it may be helpful to blanch the fruits after peeling to stop enzymatic browning in the outer pulp layer and the pulp itself. Infrared or microwave blanching treatment can be given to fruits for a limited period to inhibit the activity of enzymes that cause browning and preserve the natural color of the food (Xin et al., 2015). Maintaining the creamy white/yellow/orange color of pawpaw pulp during processing is a critical step because when the pulp browns, it may no longer be appealing to consumers. Enzymatic browning causes a decline in favorable sensory attributes during processing and storage making it the second major cause of quality loss in fruits and vegetables (Ioannou & Ghoul, 2013; X. Zhang et al., 2020).

The pulp of the Shenandoah fruits studied had a lighter color, but a redder color and a more yellow color compared to those reported by Zhang et al. (2017). Further, the pulp

from the Overleese fruits had a darker color but similar redness and yellowness compared to the Overleese pulp data reported by Brannan et al. (2015). This data suggests that freeze-thaw cycles coupled with variations in soil and environmental conditions can affect the color of pawpaw pulp in different ways depending on the fruit cultivar. Analysis of pawpaw pulp kept in frozen storage shows that over time, the frozen pulp is darker and more yellow compared to the fresh pulp (Brannan & Wang, 2017) as observed in the data obtained for the Shenandoah and the Overleese fruits studied.

3.4.3 Physicochemical and thermal properties of pawpaw pulp

In this study, the acidity of pawpaw fruits was determined by measuring both the pH and titratable acidity of the pawpaw pulp. A study by Nam et al. (2018) shows that pawpaw fruit contains acetic, formic, oxalic, malic, and citric acids, with acetic acid being the predominant acid. Freshly harvested pawpaw fruits have a pH of 6.5, however, as ripening progresses, the acidity increases and then decreases to a pH of 5.2 after 8 weeks of cold storage (Galli et al., 2008). Further, Francino (2019) reported that pawpaw fruits less ripened tend to have a higher pH. The pH values obtained in this study are similar to the values obtained by Galli et al. (2008) but higher than the pH values obtained for ripe fruits (Davis cultivar) by Donno et al. (2014). Nonetheless, the mango fruits tested had a pH of 4.40±0.29, hence, more acidic than the pawpaw fruits. To successfully use pawpaw fruits in food applications such as jams, jellies, and wine, which require high acidity, more acid would need to be added in the pawpaw preparation to achieve a similar acidity and gel formation as in the mango preparation. Also, the low acidity (almost neutral pH) of the pawpaw pulp may be another contributing factor to its rapid browning on exposure to air.

Studies have shown that acidifying agents such as ascorbic acid and citric acid can lower pH and inhibit the action of PPO, slowing enzymatic browning in fruits (Moon et al., 2020). In pawpaw pulp, studies have demonstrated that lowering the pH of the pulp with ascorbic acid has the potential to inhibit significant color changes for up to 45 days of frozen storage (Zhang et al., 2017).

Fruit juice content is an indicator of fruit maturity. Generally, the juice content in fruits increases as the fruit matures and then declines after the fruit has reached full maturity (Lado et al., 2014). The results obtained suggest that among the cultivars examined, Overleese fruits may be the best for fruit juice applications of the pawpaw fruit. It is also important to note that all the Overleese fruits used in this study were from only the Upper orchard. The percentage juice contents obtained were higher than the values reported for orange, sweet lime, lemon, and grapes by Jamil et al. (2015) although pawpaw pulp has a thicker consistency and about the same moisture content. The high juice contents obtained for the pawpaw fruits studied may be a result of changes that occurred in the fruit during thawing before analyses. Also, the differences in the soil conditions of the Lower and Upper orchards did not have a clear effect on the juice contents of the fruits in the Lower and Upper orchards. Despite this, industrial pawpaw juice extraction may require the use of mechanical juice extractors that can handle the fruit's thick consistency. Alternatively, enzymatic treatment may need to be used in pawpaw pulp prior to juice extraction since pulp treatment with enzymes like pectin methyl esterase and polygalacturonase has been shown to ease juice extraction and increase fruit juice yield in various fruits (Sharma et al., 2017).

Fruit density is often used to predict chemical composition such as dry matter, soluble solids, starch content, and physical disorders (Aubert et al., 2019). Also, the density of the fruit can be used to predict the thermophysical properties of the fruit, which will be useful during its cold storage and processing. In a study that assessed the relationship between fruit density and quality characteristics, denser fruits contained more sugar, polyphenols, and volatile compounds (Aubert et al., 2019). Based on the similarities in the pulp densities of pawpaw pulp and mango pulp, the cold storage conditions used for the storage of mango pulp may be applied for the storage of pawpaw pulp, though they may have different thermal diffusivities due to differences in specific heat capacity and thermal conductivity.

The progression of ripening in pawpaw leads to an increase in the total soluble solids (TSS) and the release of flavor volatiles (Brannan et al., 2015). The TSS of all the cultivars studied were lower than the data reported by Lolletti et al. (2021) for NC1 and Taylor cultivars but similar to the data reported for the Sunflower cultivar. It is possible that the freeze-thaw cycle could have influenced the TSS of the pawpaw fruit since it has been shown to significantly alter the TSS of some fruits (Chassagne-Berces et al., 2010). However, the effect of the freeze-thaw cycle on the TSS of the pawpaw fruit is unclear. Further studies need to be conducted to clearly understand the effect of freeze-thaw cycles on the TSS of pawpaw fruits. Studies have shown that TSS concentration has a significant effect on the inactivation of PPO in a high-pressure processing treatment. Enzymes such as polyphenol oxidases and peroxidases in fruits with higher TSS concentrations have some resistance to inactivation in high-pressure processing treatment (Kaushik et al., 2015; Zhang et al., 2017). Hence, in high-pressure processing of pawpaw fruits to inactivate the

PPO and other enzymes that cause browning, Susquehanna fruits may require more pressure to achieve the same level of enzyme inactivation as the PA Golden fruits.

The thermal properties of fruit pulp are critical for designing processing operations that involve heating and/or cooling. Also, since high-pressure processing has been suggested to be a suitable technology for extending the shelf life of pawpaw (Zhang et al., 2017), obtaining the thermal properties of the fruit pulp is very important as these parameters are essential for designing the processing operation (Juliano et al., 2011). The melting temperature of the ice in frozen pawpaw pulp obtained in this study may help improve the storage and processing conditions of pawpaw to make the fruit easier to commercialize. In future studies, investigating other thermal properties like the thermal conductivity, specific heat capacity and enthalpy at different temperatures would be helpful in better understanding the heating and cooling behaviors of pawpaw pulp.

3.4.4 Effect of freezing on pawpaw fruits and microstructural properties

In our study, we observed that parts of the fruit pulp had a rubbery texture, while other parts had a fibrous texture. To confirm our observations, SEM analysis of pulp samples taken closer to the seeds of the fruit showed a smooth, almost regular surface with no fibers. Meanwhile, SEM analysis of pulp samples taken further from the seeds revealed that a portion of the pulp was fibrous with polygonal and irregular structures on the surface. While this could have been as a result of chill injury leading to changes in the microstructure of the pulp, these structural differences may likely be due to compositional differences between those two parts of the fruit. Studies have shown that portions of fruits with high concentrations of starch or pectin tend to exhibit similar polygonal and irregular

surface morphologies as observed in the SEM images of the pawpaw pulp samples taken further from the seeds (Quirós-Sauceda et al., 2019; Wongkaew et al., 2020).

Storage temperature has been demonstrated to affect the quality characteristics of fruits. Obenland et al. (2011) demonstrated that mandarins stored at lower temperatures had a reduced flavor quality, and high soluble solids concentration to titratable acidity ratio with an increased soluble solids concentration. It is possible that the freezing temperature at which pawpaw fruits were stored before the analyses could have affected the soluble solids, acidity, and other quality characteristics. Further, visual observations made during the experiments show that the pawpaw fruits had undergone chill injury during the frozen storage period. Galli et al. (2009) indicated that the loss of antioxidant protective systems (a system that involves enzymes and antioxidants such as reduced glutathione and total ascorbate) during prolonged low-temperature storage significantly promotes chill injury in pawpaw fruits. Therefore, it is critical to optimize the frozen storage of pawpaw fruits considering the volumetric enthalpy changes (ΔH_1 and ΔH_2), Biot's number (N_{Bi}), initial temperature, final center temperature (T_a), and mean freezing temperature (T_{fm}) as shown in Pham's equations below, to adequately store pawpaw fruits, where t is the freezing time, d is a characteristic dimension (radius), h is the convective heat transfer coefficient and E_f is the shape factor (Singh & Heldman, 2009).

$$T_{fm} = 1.8 + 0.263T_c + 0.105T_a$$

$$t = \frac{d}{E_f h}\left[\frac{\Delta H_1}{\Delta T_1} + \frac{\Delta H_2}{\Delta T_2}\right]\left(1 + \frac{N_{Bi}}{2}\right)$$

Using these equations, a better cold storage system can be designed or adapted for the storage of pawpaw fruits to help retain quality attributes. To have a better understanding of the fruit devoid of the influence of chill injury, there is a need for further studies on fresh pawpaw samples.

3.5 Conclusion

The findings presented in this paper show that there are variations in the physical properties of frozen fruits from the eight pawpaw cultivars studied. Among the cultivars studied, Susquehanna fruits had the highest total fruit weight, pulp weight, volume, and total soluble solids concentration. This could potentially make Susquehanna fruits the preferred cultivar for pawpaw pulp processing. However, it is important to consider all other quality characteristics of the fruits when processing to produce a desirable high-quality product. Further, fruits of the Susquehanna cultivar had the highest fruit length, fruit width, and fruit thickness; nonetheless, these dimensions were found to be similar to mangoes, suggesting the fruit peelers designed for other fruits with similar shape and size like mangoes may be suitable for peeling pawpaw for industrial processing. Due to the pawpaw fruits' thinner peels, such fruit peelers may need to be optimized to reduce pulp wastes during pawpaw peeling. It is likely the peel thickness, peel color, and pulp color of the fruits were influenced by the freeze-thaw cycle as well as the soil and environmental variations but to different extents for the different cultivars and orchards. Fruits of the Wells cultivar may be less susceptible to bruising since they had the thickest peels of the cultivars studied. This might make them more suitable for the fresh pawpaw markets. Also, the fruits of the Sunflower cultivar had the highest peel yellowness and peel lightness. These color indicators may be helpful for farmers who plan to grow pawpaw fruits for fresh

fruit markets to be sold in grocery shops; nonetheless, due to the fruit's rapid browning, the appropriate storage mechanisms must be applied to make high-quality fresh fruits available to consumers. Overall, since pawpaw pulp has an almost neutral pH, it would be necessary to acidify the pulp or use high-pressure processing to inhibit the enzymatic browning that occurs in the pulp during storage. Juice extraction from pawpaw fruits may be more feasible with Overleese fruits than fruits from other cultivars. Potentially, the use of enzymatic treatments could ease juice extraction from all the pawpaw cultivars, and the pulp could also be used in other food applications including jams and jellies. These findings set the stage for further studies on fresh pawpaw fruits since this study was carried out with frozen samples. This will provide further understanding to develop effective postharvest loss prevention strategies and extend the shelf life of pawpaw fruits. Also, due to the diversity of genetics, there is no perfect fruit suitable for all purposes. Hence, it might be necessary to develop cultivars for specific purposes, such as cultivars for fresh fruit marketing and cultivars for fruit processing, to further ease the commercialization of the fruit.

Brief Synopsis: Chapter 4 presents the color and textural properties of unripe and ripe North American pawpaw fruits. This chapter also contains correlational analysis between color, textural properties and ripening of the North American pawpaw fruit. The findings in this chapter have been published in the *Sustainable Food Technology* journal.

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Chapter 4

Correlations between color, textural properties and ripening of the North American pawpaw (Asimina triloba) fruit

Abstract

The North American pawpaw (*Asimina triloba* [L.] Dunal) fruit is the largest edible fruit native to the United States. Over the years, the fruit has remained underutilized with limited published data on the properties of the fruit. In this study, the color of the skin and the pulp of the fruit, as well as the textural properties of unripe and ripe fruit harvested from wild trees were evaluated. The results show statistically significant differences in the textural properties of the unripe and ripe fruits. The ripe fruits had a hardness of 2.2±0.5 kg-force, similar to the hardness of green ripe mangoes but harder than green ripe bananas, and the unripe fruits had a hardness of 68.2±10.9 kg-force. Also, there were strong negative correlations between the fruit skin color a* values and the hardness (r = -0.87), chewiness (r = -0.86), and cohesiveness ratio (r = -0.73), and a strongly positive correlation with total soluble solids (r = 0.90). The skin hue angles had strong positive correlations with hardness (r = 0.86), cohesiveness ratio (r = 0.74) and chewiness (r = 0.86), and a strongly negative correlation with total soluble solids (r = -0.91). The fruit skin color a* values (degree of greenness), skin hue angle and total soluble solids content can be used as a non-invasive indicator of pawpaw ripeness. The correlations established in this study provide new insights, farmers could use commercially available portable color and near-infrared Brix meters to determine the maturity of pawpaw fruits. These findings will help farmers and

processors to harvest and process pawpaw fruits at the right time to minimize postharvest losses.

4.1 Introduction

The North American pawpaw (*Asimina triloba* [L.] Dunal) is a unique member of the Annonaceae family that grows in the temperate region of the world. The fruit of the pawpaw tree is known to be the largest edible fruit native to the United States. The pawpaw fruit has remained underutilized primarily due to its short shelf life, and the darkening of the skin after harvesting (Donno et al., 2014; Pomper & Layne, 2010). To date, the fruit has not been commercialized or standardized as a horticultural crop. Several cultivars of the North American pawpaw have recently been selected for their excellent fruit characteristics (Pomper et al., 2009), however, no objective ripeness indicators have been developed, such as those established for fruits like banana, mango, and apple (Lang & Hübert, 2012; Mazen & Nashat, 2019; Ratprakhon et al., 2020).

Unlike climacteric fruits like banana which can be harvested unripe and allowed to ripen over time, pawpaw fruits are best harvested when ripe. Unripe pawpaw fruits have been found to remain unripe even after comingling with ripe pawpaw fruits, suggesting a low sensitivity to ethylene (Archbold et al., 2003). To harvest pawpaw fruits, the pawpaw tree is given a gentle shake to allow ripe fruits to fall by themselves to the ground. During ripening, the fruit peduncle (portion linking the fruit to the stem of the tree) softens leading to the fall of fruit from the tree when mature. Further, the ripening of the fruit has been reported to be characterized by a loss of the green color intensity, reduction in the hardness, and increases in the soluble solids content and volatile aroma compounds (Archbold et al.,

2003; Mcgrath & Karahadian, 1994). However, the correlations between noninvasive ripeness indicators like color and invasive indicators like texture and soluble solids content have not been studied to confirm the general notion that color is not a good indicator of pawpaw fruit ripeness.

This research aims to investigate the textural properties and the color of both ripe and unripe pawpaw fruits, and to test the correlations between the color, textural properties, and total soluble solids content to gain insights on the potential use of noninvasive indicators for pawpaw fruit ripeness. These insights into the color and textural properties of ripening pawpaw fruits will be helpful to identify attributes to monitor fruit ripeness to prevent fruit loss as interest in the crop increases.

4.2 Materials and Methods

4.2.1 Fruit Samples

Ripe and unripe pawpaw fruits were harvested from eight-year-old seedling trees of wild origin growing in a butterfly garden near Eckles Hall on the University of Missouri campus, Columbia, Missouri. In August 2021, ripe pawpaw fruits were harvested by gently shaking the branches of the trees, while nearly mature but still unripe fruits were plucked from the same trees on the same day. The fruits were immediately brought to the laboratory for analysis.

4.2.2 Fruit color

Fruit color was measured according to the method described by Nambi and others (Nambi et al., 2015) using the Hunter LAB color meter (Chroma Meter CR-410, Konica Minolta). The analyses were carried out with 13-17 fruits each for unripe and ripe fruits.

Five readings for each pawpaw skin and five readings for each pawpaw pulp were read at five different places on each fruit. The averages for the recordings were used to calculate the total color difference (ΔE or Delta E), chroma, and hue angle using the equations below where, L* is the degree of lightness to darkness, a* is the degree of redness to greenness, b* is the degree of yellowness to blueness, the subscripts f and i denote final (ripe fruits) and initial (unripe fruits) value.

$$\Delta E = \sqrt{(L*_f - L*_i)^2 + (a*_f - a*_i)^2 + (b*_f - b*_i)^2} \ldots (1)$$

$$Chroma = \sqrt{a*^2 + b*^2} \ldots (2)$$

$$Hue\ Angle = tan^{-1}\left(\frac{b*}{a*}\right) \ldots (3)$$

Hue angle values were corrected for the respective quadrants, where 0°/360° = red, 90° = yellow, 180° = green and 270° = blue as described by McLellan and others (McLellan et al., 1995).

4.2.3 Total soluble solids

Total soluble solids (TSS) content was measured according to the AOAC Official Method 932.14C (AOAC, 2000b) using a digital refractometer (HI96800, Hanna Instruments) at ~25ºC. Thirteen ripe fruits were used for this analysis. A sample of the ripe pulp was placed in the sample well of the refractometer. The total soluble solids measurements were taken in triplicates for each fruit and recorded as degree Brix. The total soluble solids content of the unripe fruits was not measured because the pulp was hard.

4.2.4 Texture Analyses

Textural properties of the fruits were determined by a Texture Profile Analysis (TPA) following the method described by Yang and others (Yang et al., 2007) with some modifications. Pawpaw fruits were analyzed for their hardness, chewiness, cohesiveness, springiness ratio (or springiness) and resilience ratio using a Texture Analyzer (TA.HDPlus C, Stable Micro Systems) equipped with a 100kg load cell and connected to the Exponent Connect Software (Stable Micro Systems). A P/75 (3-inch diameter) compression plate was used for the analyses. The texture analyzer was programmed to carry out a texture profile analysis with the following test conditions: pretest speed of 1mm/sec, test speed of 1mm/sec, post-test speed of 1mm/sec, trigger force of 5g, compression distance of 10mm and a time of 5sec between compressions. The analyses were carried out with 13-17 fruits each for unripe and ripe fruits of similar size and shape. An illustration of the texture profile with the variables used to obtain the textural parameters are shown in Figure 4-1. Hardness and chewiness were recorded in kilograms of force (kg); cohesiveness and resilience ratios were recorded as percentages (%), and springiness ratio was recorded as a dimensionless ratio.

$$Chewiness = hardness \times cohesiveness \times springiness \ldots (4)$$

$$Cohesiveness\ ratio = \frac{Area\ 2}{Area\ 1} \times 100 \ldots (5)$$

$$Resilience\ ratio = \frac{Area\ 4}{Area\ 3} \times 100 \ldots (6)$$

$$Springiness\ ratio = \frac{Distance\ 2}{Distance\ 1} \ldots (7)$$

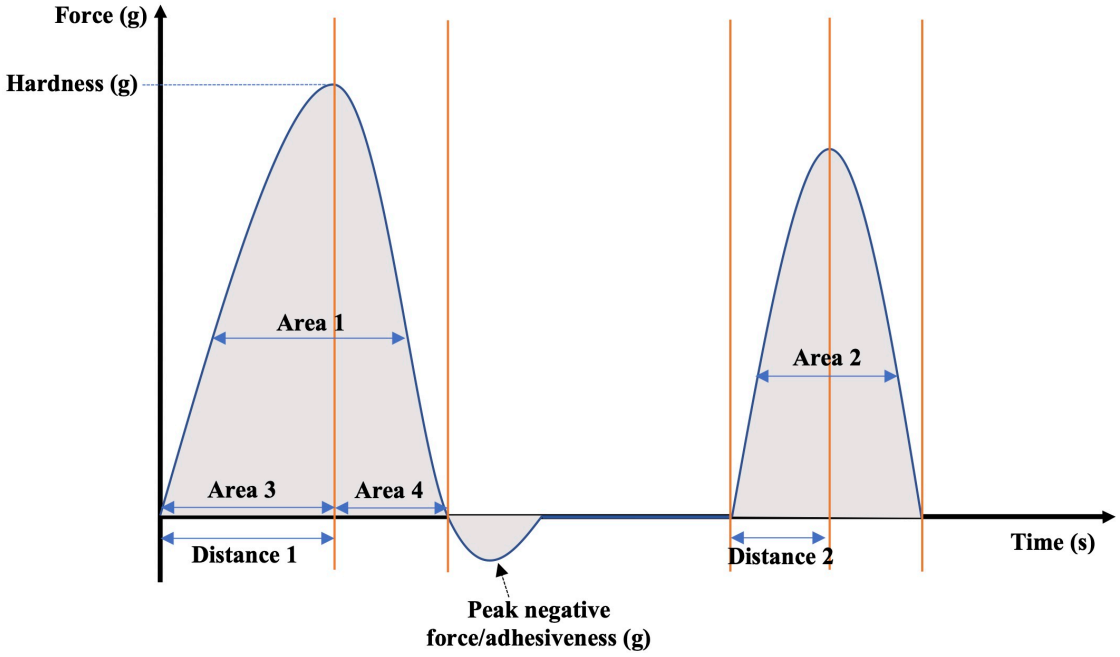

Figure 4-1: An illustration of a texture profile from which the hardness, chewiness, cohesiveness ratio, resilience ratio, and springiness ratio are obtained.

4.2.5 Statistical Analyses

Student's t-test was used to test for differences in the means of the textural properties and color indices of the unripe and ripe pawpaw fruits. The data were analyzed at a significance level of 0.05 using JMP 14.0.0 software (SAS Institute Inc., Cary, USA). Principal component analysis (PCA), hierarchical cluster analysis (HCA) and Pearson's correlation analysis were performed using OriginPro 2021 version 9.8.0 software (Origin Lab Inc., Northampton, Massachusetts, USA).

4.3 Results

4.3.1 Color of fruit and pulp

The data obtained show that apart from the b* values, there were significant differences in all the color parameters between unripe and ripe fruits (Table 4-1). The unripe fruits had higher L* values (59.9±3.5) than the ripe fruits (56.2±5.4) ($p=0.0002$) indicating the unripe fruits were lighter in color than the ripe fruits. The unripe fruits recorded a high skin a* value (degree of greenness) (-15.2±4.6) whereas the ripe fruits showed some degree of redness (b* value) on the skin (0.9±4.9). The unripe fruits had a slightly higher color saturation (chroma) than the ripe fruits as shown in Table 4-1 and Figure 4-2. Also, the data obtained show that there is a clear visible total color difference (Delta E) in the skin colors of the unripe and ripe fruits (18.4±4.2) (Table 4-1). The hue angles for the colors of the fruits indicated that the average color of the unripe fruits (118.8°±3.9°) lies between yellow (90°) and green (180°) while the average color of the ripe fruits (87.4°±9.1°) lies between red (0°) and yellow (90°) (Table 4-1). The color data for the unripe and ripe pulps show a more significant difference in the lightness (L*) and color saturation (chroma) values of the fruit pulp (Table 4-1).

Table 4-1: External fruit (skin) color and pulp color of pawpaw fruits grown in central Missouri, 2021

	L*	a*	b*	Chroma	Delta E	Hue Angle (°)
Unripe Fruit Skin	59.9±3.5	-15.2±4.6	27.5±3.2	31.7±3.4	0.0±0.0[1]	118.8±3.9
Ripe Fruit Skin	56.2±5.4	0.9±4.9	27.8±5.7	28.2±5.8	18.4±4.2	87.4±9.1
t-test	3.85	-15.83	-0.27	3.52	-28.90	-10.45
p-value	0.0002	<0.0001	0.7863	0.0007	<0.0001	<0.0001
Unripe Pulp	76.2±3.9	-7.2±1.8	24.9±2.9	26.0±3.1	0.0±0.0[1]	106.1±2.9
Ripe Pulp	63.0±3.7	11.6±2.1	50.1±6.3	51.5±6.5	34.5±5.5	77.0±1.8
t-test	16.42	-44.74	-23.98	-23.50	-41.43	-28.26
p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

All t-tests were two-tailed with 0.05 level of significance

[1]Delta E values calculated based on difference from unripe samples

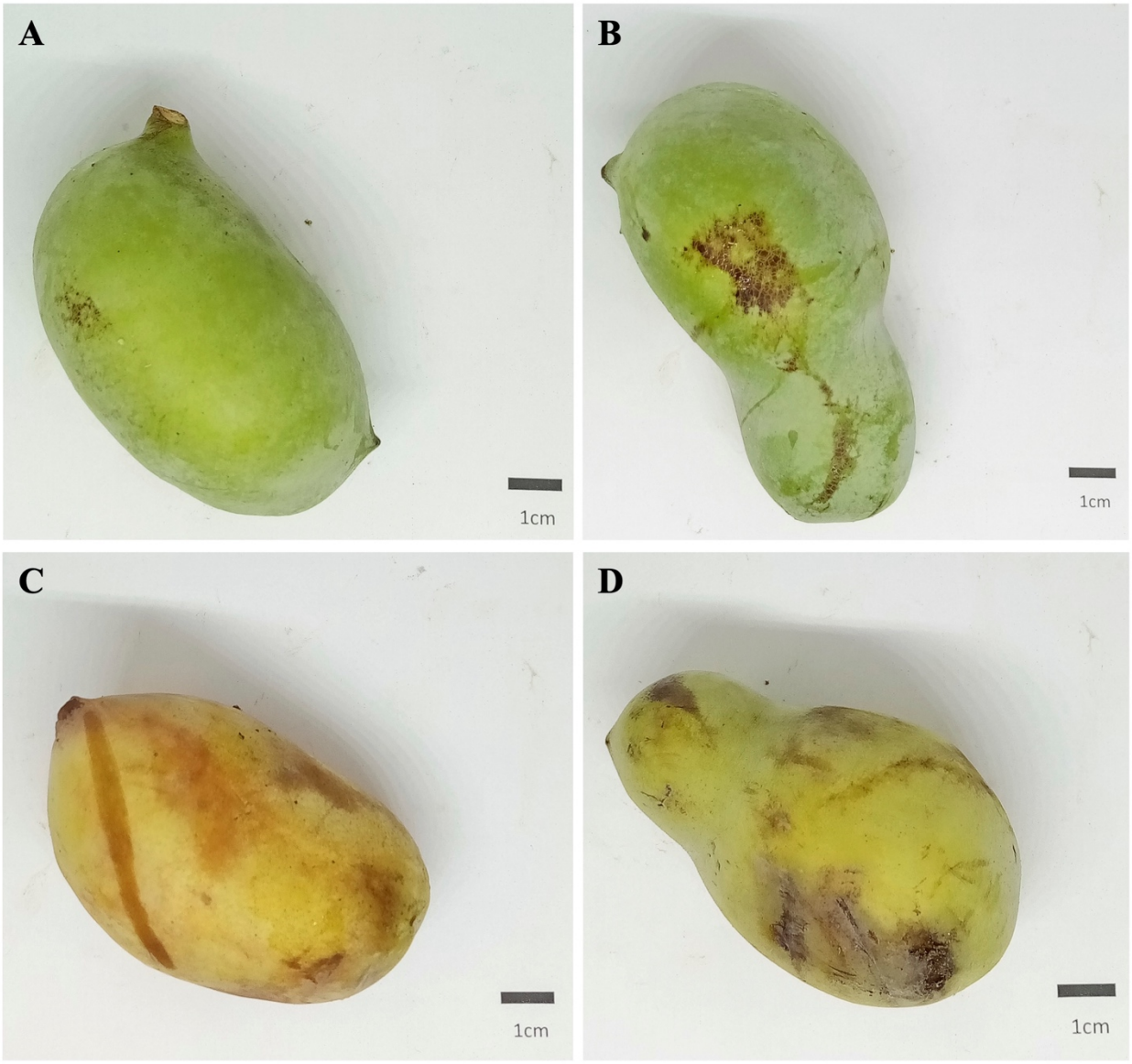

Figure 4-2: Images of unripe pawpaw fruits (A & B) and ripe pawpaw fruits (C & D)

4.3.2 Total soluble solids content

The total soluble solids content (Brix) of the pulp of the ripe fruits was 21.52±0.94. The total soluble solids content of the unripe fruits was not measured due to its hard texture, which made it difficult to determine the total soluble solids content.

4.3.3 Texture profile of fruits

The hardness of the unripe pawpaw fruits (68.2±10.9 kg-force) was significantly higher than that of the ripe fruits (2.2±0.5 kg-force) (Table 4-2) as shown in Figure 4-3. The data obtained in this study shows that the unripe fruits had a significantly high cohesiveness (56.9±8.1%) compared to the ripe fruits (19.7±2.4%) (Table 4-2). The unripe fruits had a chewiness of 167.6±20.4 kg-force while the ripe fruits recorded a chewiness of 1.7±0.6 kg-force (Table 4-2). Unripe fruits (27.4±2.9%) had a significantly higher resilience ratio than the ripe fruits (7.5±1.3%), however, the springiness of the unripe fruits (3.6±0.7) was not statistically different from that of the ripe fruits (3.5±0.2).

Table 4-2: Textural properties of unripe and ripe pawpaw fruits grown in central Missouri, 2021

	Hardness (kg-force)	Chewiness (kg-force)	Cohesiveness Ratio (%)	Resilience Ratio (%)	Springiness Ratio
Unripe Fruit	68.2±10.9	167.6±20.4	56.9±8.1	27.4±2.9	3.6±0.7
Ripe Fruit	2.2±0.5	1.7±0.6	19.7±2.4	7.5±1.3	3.5±0.2
T-test	10.12	12.66	7.69	3.14	1.49
p-value	<0.0001	<0.0001	<0.0001	0.004	0.148

All t-tests were two-tailed with 0.05 level of significance.

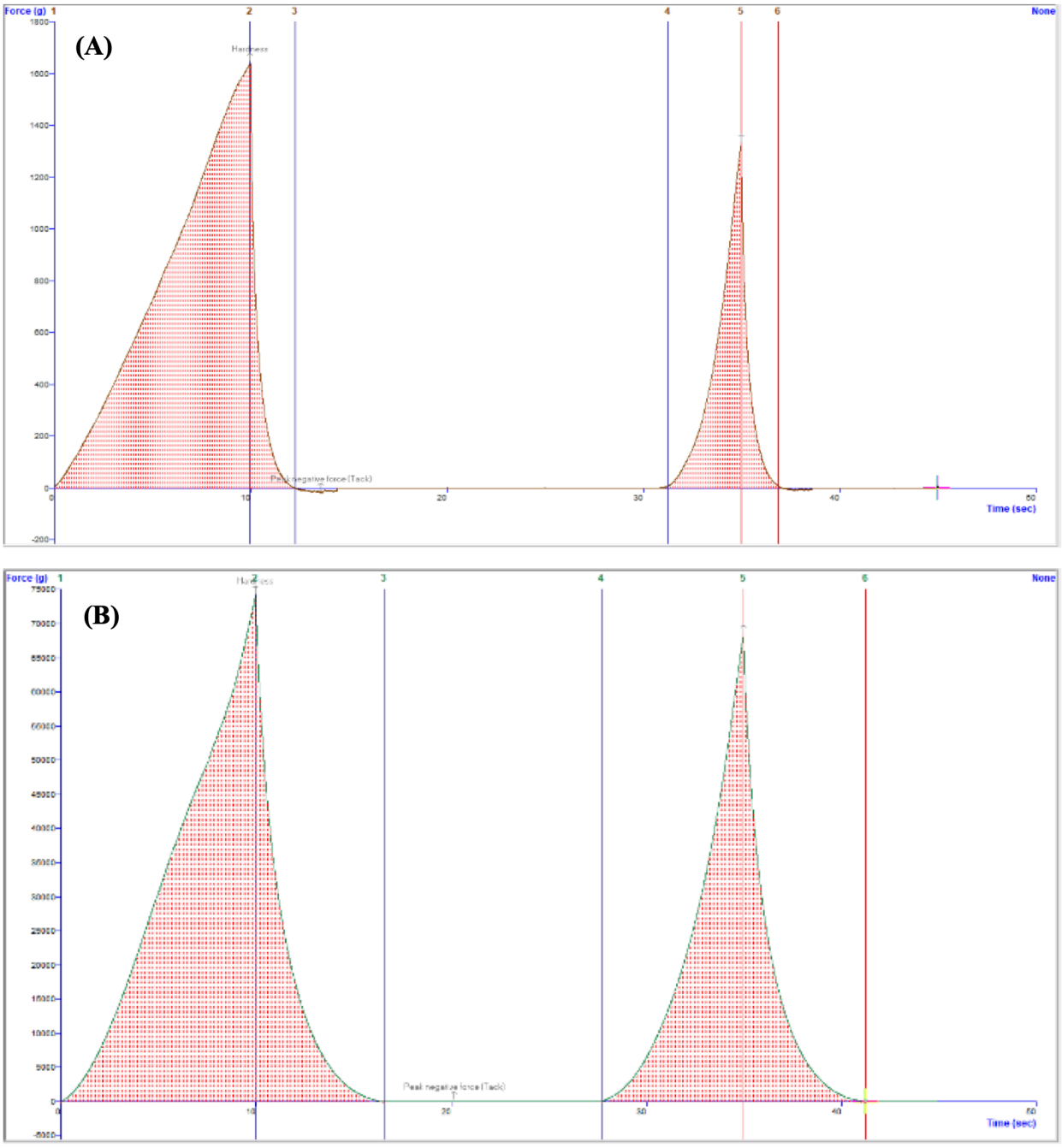

Figure 4-3: Texture profiles of (A) ripe pawpaw fruit and (B) unripe pawpaw fruit

4.3.4 Pearson's correlation analysis

From the correlation plot and correlation matrix (Figure 4-4 and Table 4-3), statistically significant correlations between the skin and pulp color parameters and the textural properties were found. Most of the stronger correlations were between the pulp color and the textural properties. The correlation matrix shows that there is a strong negative correlation between pawpaw fruit hardness and the skin a* values ($r = -0.87$, $p \leq 0.01$), and fruit hardness and skin total color difference ($r = -0.87$, $p \leq 0.01$), and a strong positive correlation between fruit hardness and skin hue angle ($r = 0.86$, $p \leq 0.01$). Also, the cohesiveness ratio had strong negative correlations with the skin a* value ($r = -0.73$, $p \leq 0.01$) and the skin total color difference ($r = -0.84$, $p \leq 0.01$) but a strong correlation with skin hue angle ($r = 0.74$, $p \leq 0.01$). Similarly, chewiness had a strong negative correlation with the skin a* value ($r = -0.86$, $p \leq 0.01$) and the skin total color difference ($r = -0.88$, $p \leq 0.01$), and a strong positive correlation with the skin hue angle ($r = 0.86$, $p \leq 0.01$). Total soluble solids had a strong positive correlation with the skin a* value ($r = 0.90$, $p \leq 0.01$), skin hue angle ($r = 0.86$, $p \leq 0.01$), and the skin total color difference ($r = 0.95$, $p \leq 0.01$).

Table 4-3: Correlation matrix of Pearson's correlation coefficients (r) for the color, total soluble solids, and textural properties of pawpaw fruits grown in Central Missouri, 2021

	L* skin	a* skin	b* skin	Chroma skin	Delta E skin	Hue Angle skin	L* pulp	a* pulp	b* pulp
L* skin	1.00	-0.67	0.63	0.78	-0.62	0.65	0.49	-0.52	-0.49
a* skin	-0.67	1.00	-0.24	-0.66	0.97	-0.99	-0.80	0.89	0.88
b* skin	0.63	-0.24	1.00	0.86	-0.10	0.18	0.03	0.01	0.04
Chroma skin	0.78	-0.66	0.86	1.00	-0.56	0.61	0.49	-0.48	-0.43
Delta E skin	-0.62	0.97	-0.10	-0.56	1.00	-0.98	-0.84	0.95	0.95
Hue Angle skin	0.65	-0.99	0.18	0.61	-0.98	1.00	0.80	-0.90	-0.90
L* pulp	0.49	-0.80	0.03	0.49	-0.84	0.80	1.00	-0.89	-0.84
a* pulp	-0.52	0.89	0.01	-0.48	0.95	-0.90	-0.89	1.00	0.95
b* pulp	-0.49	0.88	0.04	-0.43	0.95	-0.90	-0.84	0.95	1.00
Chroma pulp	-0.49	0.88	0.04	-0.43	0.95	-0.90	-0.85	0.95	1.00
Delta E pulp	-0.52	0.90	0.02	-0.47	0.96	-0.91	-0.90	0.99	0.98
Hue Angle pulp	0.50	-0.89	-0.02	0.47	-0.95	0.90	0.87	-0.99	-0.94
Hardness	0.44	-0.87	0.07	0.52	-0.87	0.86	0.79	-0.91	-0.85
Resilience ratio	0.29	-0.33	-0.11	0.15	-0.47	0.34	0.49	-0.48	-0.46
Cohesiveness ratio	0.44	-0.73	-0.09	0.36	-0.84	0.74	0.81	-0.85	-0.83
Springiness ratio	0.01	-0.24	-0.33	-0.08	-0.30	0.28	0.36	-0.27	-0.37
Chewiness	0.44	-0.86	0.00	0.47	-0.88	0.86	0.87	-0.89	-0.88
Total soluble solids	-0.45	0.90	0.07	-0.44	0.95	-0.91	-0.89	0.98	0.95

Table 4-3: Correlation matrix of Pearson's correlation coefficients (r) for the color, total soluble solids, and textural properties of pawpaw fruits grown in Central Missouri, 2021

	Chroma pulp	Delta E pulp	Hue Angle pulp	Hardness	Resilience ratio	Cohesiveness ratio	Springiness ratio	Chewiness	Total soluble solids
L* skin	-0.49	-0.52	0.50	0.44	0.29	0.44	0.01	0.44	-0.45
a* skin	0.88	0.90	-0.89	-0.87	-0.33	-0.73	-0.24	-0.86	0.90
b* skin	0.04	0.02	-0.02	0.07	-0.11	-0.09	-0.33	0.00	0.07
Chroma skin	-0.43	-0.47	0.47	0.52	0.15	0.36	-0.08	0.47	-0.44
Delta E skin	0.95	0.96	-0.95	-0.87	-0.47	-0.84	-0.30	-0.88	0.95
Hue Angle skin	-0.90	-0.91	0.90	0.86	0.34	0.74	0.28	0.86	-0.91
L* pulp	-0.85	-0.90	0.87	0.79	0.49	0.81	0.36	0.87	-0.89
a* pulp	0.95	0.99	-0.99	-0.91	-0.48	-0.85	-0.27	-0.89	0.98
b* pulp	1.00	0.98	-0.94	-0.85	-0.46	-0.83	-0.37	-0.88	0.95
Chroma pulp	1.00	0.98	-0.94	-0.85	-0.46	-0.83	-0.38	-0.88	0.95
Delta E pulp	0.98	1.00	-0.98	-0.89	-0.48	-0.86	-0.34	-0.90	0.98
Hue Angle pulp	-0.94	-0.98	1.00	0.91	0.49	0.85	0.28	0.89	-0.99
Hardness	-0.85	-0.89	0.91	1.00	0.26	0.69	0.17	0.90	-0.90
Resilience ratio	-0.46	-0.48	0.49	0.26	1.00	0.84	0.25	0.48	-0.50
Cohesiveness ratio	-0.83	-0.86	0.85	0.69	0.84	1.00	0.41	0.83	-0.87
Springiness ratio	-0.38	-0.34	0.28	0.17	0.25	0.41	1.00	0.50	-0.35
Chewiness	-0.88	-0.90	0.89	0.90	0.48	0.83	0.50	1.00	-0.92
Total soluble solids	0.95	0.98	-0.99	-0.90	-0.50	-0.87	-0.35	-0.92	1.00

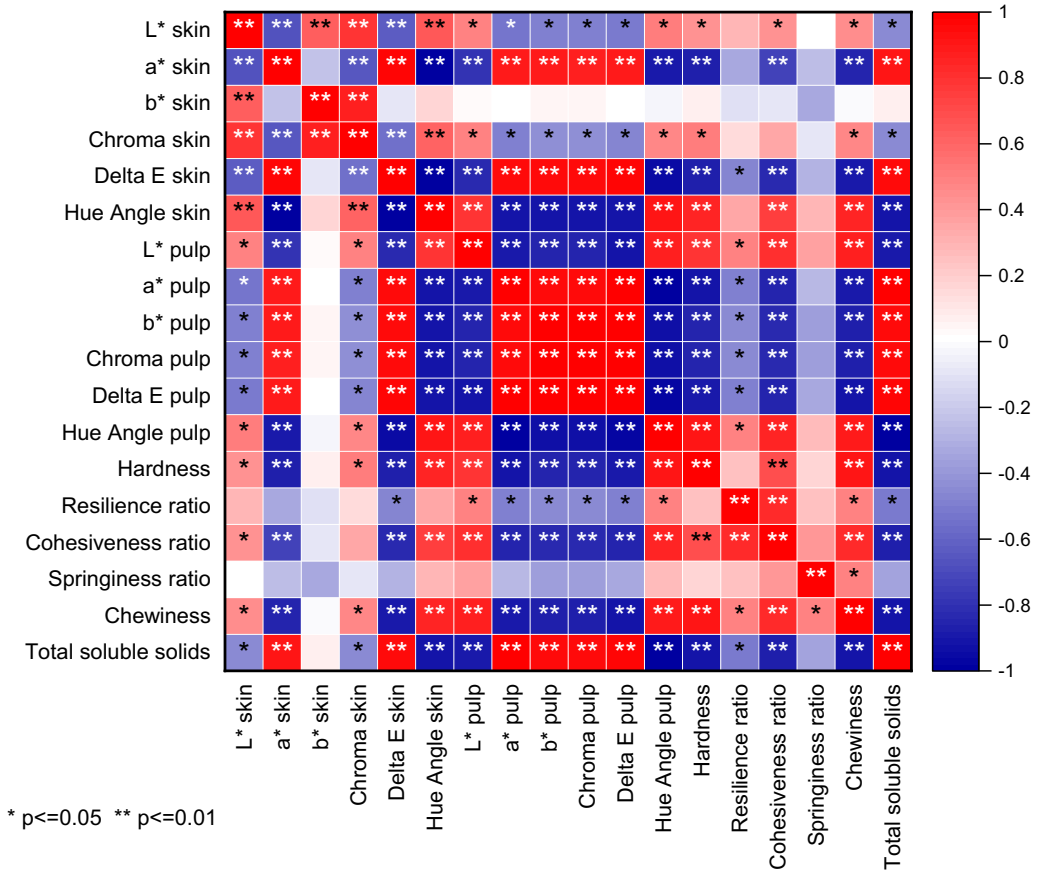

Figure 4-4: Correlation plot showing statistical significance for Pearson's correlations among the color, total soluble solids, and textural properties of pawpaw fruit.

4.3.5 Multivariate analysis

From the PCA results (Figure 4-5A), the variance contribution rates of the first, second, and third PCs (principal components) are 70.86%, 13.57%, and 5.76% respectively.

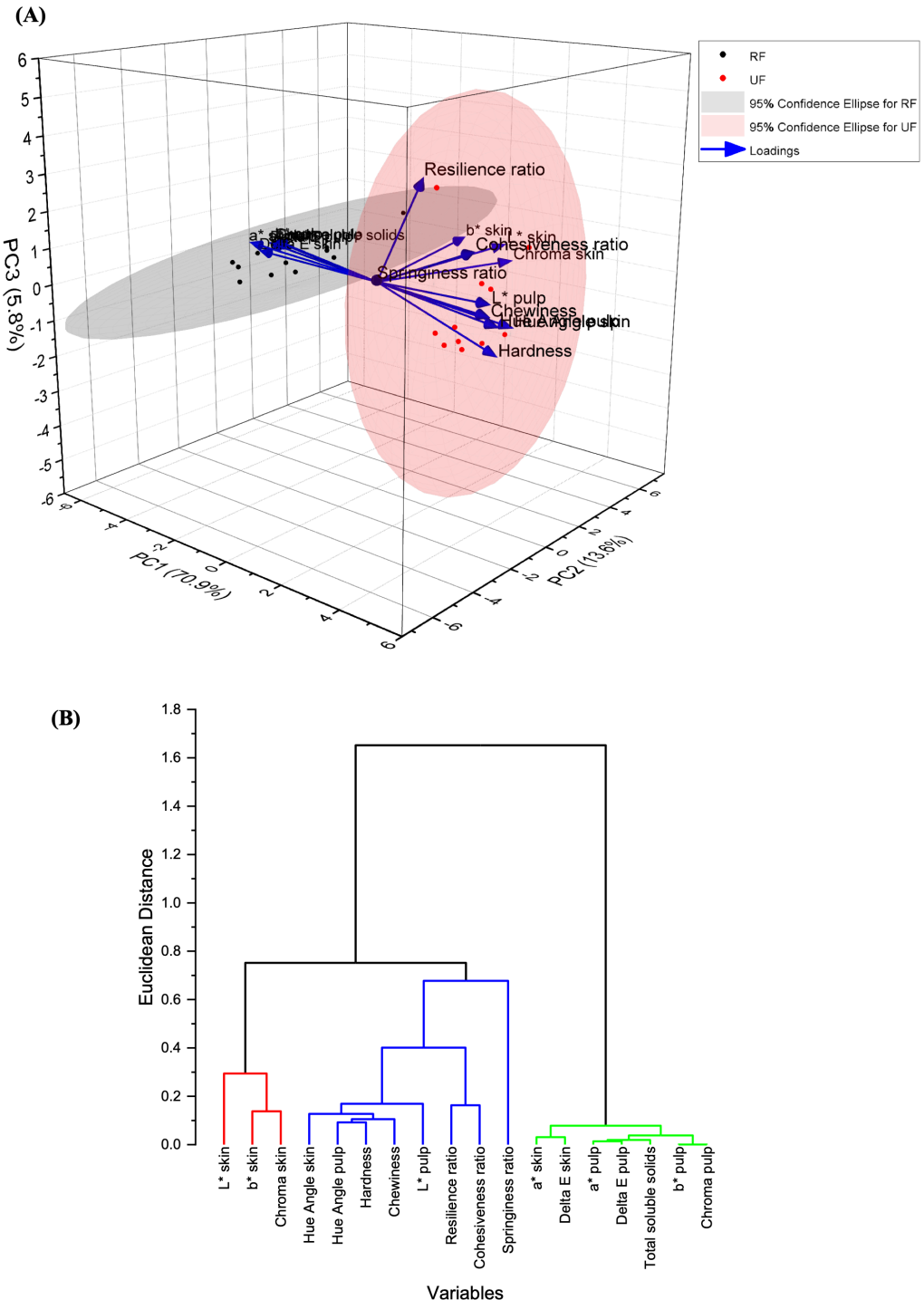

Figure 4-5: (A) Principal component analysis biplot and (B) hierarchical cluster analysis dendrogram of the ripe and unripe pawpaw fruits based on their fruit and pulp color, total soluble solids, and textural properties.

4.4 Discussion

4.4.1 Color of fruit and pulp

The color of the pawpaw fruit and pulp was determined using the Hunter L, a, b color system. The data obtained were used to calculate the chroma, which is an indication of color saturation; total color difference using the unripe color data as the initial data and the ripe color data as the final data; and hue angle, which shows the quadrant the color of the sample lies in within two-dimensional space (McLellan et al., 1995; Yang et al., 2007). The unripe fruits had higher L* values than the ripe fruits because as pawpaw fruits ripen their skin darkens. Further, the darkening of the skin becomes intense in the first few hours after harvesting if the fruits are bruised. The darkening of the ripe fruits could also be attributed to the method of harvesting, which involved allowing the fruits to fall to the ground, possibly resulting in some mechanical damage thereby affecting the lightness of the ripe fruits. Since negative a* values indicate green color and positive a* values indicate red color, it is clear that the unripe fruits have a greener skin color compared to the ripe fruits. The skin color of the ripe fruits studied was lighter, and greener than those obtained by Adainoo and others (Adainoo et al., 2022) in a study in which ripe fruits had been frozen for weeks before analyses.

The slightly higher chroma of the unripe fruits may be a result of the darkening in the skin of the ripe fruits from the sustained bruises during harvesting (Figure 4-1). In addition, the total color difference (Delta E) shows the degree to which the color of the ripe fruits differs from that of the unripe fruits. Delta E values range from 0 to 100 with a Delta E value of 0 meaning the two colors being compared are mathematically exact and a Delta E value of 100 meaning the widest visible difference between the two colors being

compared. A Delta E value lower than 0.3 is not visible to the human eye, whereas a Delta E value of 2 is the minimum for visually detecting the difference between saturated colors (Bhookya et al., 2020). Further, the visible total color difference between the unripe and ripe fruits is shown in the hue angle data obtained. However, based on the hue angle data obtained, the variation in the color of the ripe fruits shows that a pawpaw fruit may be ripe and still have a color that lies in the same quadrant as an unripe fruit as shown in Figure 4-1D. Therefore, the skin hue angle of the fruit may be used as an index for determining the ripeness of pawpaw fruits, but it may not be a good predictor of pawpaw ripeness for all fruits. Nevertheless, previous studies have noted that as pawpaw fruits ripen, their hue angle decreases and fruits with hue angles of about 100º or lower typically have high total headspace aroma volatile contents which are indicative of ripe fruits (Mcgrath & Karahadian, 1994).

Based on the wider differences between the colors of the unripe pulp and ripe pulp compared to that of the unripe and ripe fruit, pulp color might be a better predictor of pawpaw ripeness. However, since determining the color of the pulp is invasive, it may not be a productive option for determining pawpaw ripeness. Nonetheless, the color data obtained in this experiment were consistent with the data obtained for four cultivars of pawpaw analyzed by Mcgrath and Karahadian (Mcgrath & Karahadian, 1994) and the data obtained for eight cultivars of pawpaw analyzed by Adainoo and others (Adainoo et al., 2022).

4.4.2 Texture profile of fruits

The textural properties of the fruits determined in this study were the hardness, chewiness, cohesiveness, springiness, and resilience ratio of the fruits. Hardness is given

by the maximum force of the first compression (Kasapis & Bannikova, 2017). The hardness indicates how much force must be applied to the fruit to compress it by 10 mm (based on the settings of the texture analyzer used in this study). Fruit hardness is a characteristic that is dependent on the elasticity modulus (the measure of how elastic a material is) and visco-elastic properties of the fruit (Huang et al., 2018). These physical properties are influenced by the composition of the fruit. For the pawpaw fruits analyzed, the high fiber content may explain the high hardness values for the unripe fruits (Park et al., 2022). Studies show that there is a positive linear correlation between hardness and pectin content, and a negative linear correlation between hardness and crude fiber and moisture content (Singh et al., 2013). However, there are currently no published data on the various polysaccharides in the pawpaw fruit to draw a conclusive inference. The hardness of the ripe wild pawpaw fruits used in this study was higher than the hardness of ripe 'Shenandoah' pawpaw fruits reported by Zhang and others (Zhang et al., 2017) but lower than that of the tree-ripened pawpaw fruits studied by Mcgrath and Karahadian (Mcgrath & Karahadian, 1994). Additionally, the hardness of the ripe pawpaw fruits was similar to the hardness of freshly harvested green ripe mangoes (2.5 kg-force) but higher than that of freshly harvested green ripe bananas (1.28 kg-force) (Huang et al., 2018; Omid et al., 2011).

Cohesiveness is a measure of the strength of the internal bonds that keep the food sample intact (Kamal-Eldin et al., 2020; Kasapis & Bannikova, 2017). In a TPA, cohesiveness is given by the sum of the second area of compression and retraction (A2) divided by the sum of the first area of compression and retraction (A1) expressed as a percentage as shown in the equation (5) above. The unripe fruits had a higher cohesiveness compared to the ripe fruits possibly because as the fruit ripens, the conversion of the

complex sugars into simple sugars loosens up the structure, making the ripe fruits less cohesive than the unripe fruits. In a study conducted with date fruits, it was observed that there was a positive correlation between the arabinoxylan (a polysaccharide mainly found in the cell wall of some plants) concentration and the cohesiveness of the fruits ($r=0.623$, $p<0.01$), but there was no significant correlation between the total fiber content and the cohesiveness (Kamal-Eldin et al., 2020). A similar conclusion may be drawn for the unripe pawpaw fruits, however, there is a need for further studies into the various polysaccharides present in the fruit at that stage of maturity to adequately arrive at a similar conclusion. Due to the relatively low cohesiveness of the ripe fruits, it may not be suitable to pack a lot of fruits on top of each other during transportation or at the point of sale as this may result in the deformation of the fruit, making it less appealing to consumers.

Chewiness is the energy needed to chew the fruit until it is ready to swallow (Kasapis & Bannikova, 2017). It is given by the product of hardness, cohesiveness, and springiness. While the chewiness values obtained for the ripe pawpaw fruits were within the chewiness range of apples (0.54 – 1.55 kg-force), the values obtained for the unripe fruits (167.6±20.4 kg-force) were too high above the chewiness range of apples (Guiné et al., 2011). This may be due to the presence of high levels of polysaccharides like pectin and starch which have not yet been converted into soluble sugars in the unripe fruit. In most fruits, the conversion of polysaccharides into soluble sugars during ripening is accompanied by a decrease in the amount of energy needed to chew the fruit until it is ready to swallow.

Resilience ratio is a measure of how a sample fights to return its original height after the first compression in a TPA before the waiting period starts. Results from this study clearly show that the unripe fruits have a higher resilience ratio compared to the ripe fruits.

This may have been due to the higher strength of the internal bonds in the unripe fruits as shown in the cohesiveness ratio values. The stronger internal bonds in the unripe fruits may have contributed to the recovery of the original height of the samples after the first TPA compression, whereas the weaker bonds in the ripe fruits resulted in a much lower resilience ratio. These findings are key in the packaging and transportation of pawpaw fruits to avoid deforming the fruits before they reach the target market since resilience of fruits is a key quality index among consumers (Lázaro & de Lorenzo, 2015).

Springiness is a dimensionless measure of the elasticity of a sample; it is a measure of a sample's ability to return to its original form when the force of deformation is removed. A higher springiness indicates a high sample elasticity. It is determined by the ratio between the residual displacement and maximum displacement (Li et al., 2011). The springiness ratios obtained for both unripe and ripe pawpaw fruits in this study were higher than the springiness reported for fruits like fresh blueberries (0.46-0.69), apples (0.88), and bayberry fruits (0.95) but lower than the maximum springiness ratio of date fruits (5.3) (Li et al., 2011; Najafi Marghmaleki et al., 2020; Singh et al., 2013; Yang et al., 2007). This relatively high springiness ratio of the pawpaw fruits compared to the fruits mentioned above may be largely due to the differences in fruit cell size and tissue layers (Giongo et al., 2013; McAtee et al., 2009). Further, the data obtained show that the springiness of the unripe and ripe fruits is not very different even though the unripe fruits have a slightly higher springiness ratio value. Studies in other fruits like blueberries show that springiness is often maintained during the early stages of storage after harvesting, while in other fruits like dates, springiness is highest at the moisture content of about 21.5% (Li et al., 2011; Singh et al., 2013). Since both unripe and ripe pawpaw fruits in the present study were not

stored for more than 24 hours prior to their analysis, their high springiness may have been maintained by their moisture contents. Future studies should investigate the storage conditions and the moisture contents of pawpaw fruits and how they relate to their springiness ratio and other textural properties to gain deeper insights.

4.4.3 Correlation analysis of measured quality characteristics

Skin color by itself has generally been known not to be a good indicator of pawpaw fruit ripeness. Farmers typically determine the ripeness of pawpaw fruits by their hardness (or firmness) by touch. However, since the fruits have a thin peel thickness, there is a high potential for breaking the skin through checking the hardness by touch (Adainoo et al., 2022). Currently, no research has been published testing the correlations between the skin color, which can be determined noninvasively, the textural properties, which are currently the main indices for assessing pawpaw ripeness, and the total soluble solids content (Brix). Establishing good correlations between these two physical properties and the total soluble solids contents will enable farmers to use commercially available portable devices (like handheld color meters and near-infrared total soluble solids sensors) to noninvasively determine the ripeness of pawpaw fruits. Potentially, future studies could also explore the use of unmanned aerial vehicles (drones) with artificial intelligence systems to monitor farms for pawpaw fruit skin color parameters to ensure prompt harvesting of fruits to prevent postharvest losses as has been done for fruits like strawberries and apples (Sun et al., 2020; Zhou et al., 2021).

Since it has already been established that the textural properties like hardness and cohesiveness ratio of pawpaw fruits are better indicators of ripeness, Pearson's correlation analysis was performed to test the relationship between the color parameters and the

textural properties to evaluate which color parameters correlate with the textural properties and may be used as indicators of ripeness. Studies on climacteric fruits like apples and bananas show that specific color parameters like a* values, b* values, and hue angles can be used as indicators of ripeness (Lang & Hübert, 2012; Soltani et al., 2010). According to Mcgrath and Karahadian (Mcgrath & Karahadian, 1994), b* values (degree of yellowness) are good indicators of pawpaw ripeness and not a* values. However, the findings in this study indicate otherwise. From the correlations, it can be inferred that skin a* value (degree of greenness), total color difference, and hue angle are better non-destructive indicators of pawpaw fruit ripeness compared with the other skin color indices and the pulp color parameters which are destructive. Based on this, skin a* value, total color difference, and hue angle of pawpaw fruits may be more useful in predicting the ripeness of pawpaw fruits. Skin lightness (L*) values, b* values (degree of yellowness), and chroma had relatively weak correlations with the textural properties of the pawpaw fruits, hence, these skin color parameters may not be suitable indicators of pawpaw fruit ripeness. There were strong negative and positive correlations between the pulp color parameters and the textural properties, and between the total soluble solids and the textural properties of the fruits as shown in the correlation plot and correlation matrix (Figure 4-4 and Table 4-3). However, since the determination of the pulp color and total soluble solids is destructive, they would not be productive indices for determining fruit ripeness on a large scale. This further shows the need for identification of non-invasive methods to determine the ripeness of underutilized pawpaw fruits.

4.4.4 Multivariate analysis of measured quality characteristics

The principal component analysis (PCA) was used to reduce the dimensionality of the data matrix and highlight the differences in the quality characteristics of the pawpaw fruits. The total variance contribution rate of the first three principal components (PCs) is 90.20% (more than 85%), hence, most information of the original data i.e., 90.20% of the total variance of the color, total soluble solids, and texture data, can be explained by the first three PCs (Liu et al., 2012). From the biplot, the ripe fruits are separated from the unripe fruits based on the PCA scores calculated with the color, texture, and total soluble solids data obtained in this study. The extracted eigenvectors for the PCA show that PC1 was mainly contributed to positively by skin hue angle, pulp hue angle, chewiness, pulp L* values, and hardness. On the other hand, PC1 was negatively contributed to by pulp total color difference, skin total color difference, total soluble solids, pulp a* values, and pulp b* values. For PC2, the skin b* values, skin chroma, and skin L* were the main variables that had main positive contributions; whereas springiness ratio, resilience ratio, cohesiveness ratio, and skin a* values were the main variables that negatively contributed to PC2. PC3 was mainly positively contributed to by the resilience ratio, cohesiveness ratio, skin L* values, and springiness ratio, while it was mainly negatively contributed to by the hardness and the skin hue angle. In the PCA biplot, the unripe fruits (UF) appeared mostly on the positive side of PC1 and PC2 while the ripe fruits (RF) appeared mostly on the negative side of PC1, with some on the positive side of PC3. This shows that largely, there are clear differences between ripe and unripe pawpaw fruits based on their textural and color characteristics.

Hierarchical cluster analysis (HCA) was applied using Euclidean distance as a measure of similarity, and the dendrogram showed three distinct clusters, enabling a good classification of the properties to determine the ripeness of pawpaw fruits (Figure 4-5B). The skin a*, skin total color difference, pulp a*, pulp total color difference, total soluble solids, pulp b*, pulp chroma, and skin hue angle were grouped in the same cluster, indicating the close relation of these quality indices in showing the ripeness of pawpaw fruits. This confirms the results from the PCA since these same quality indices were closely packed together on the negative side of PC1 in the score plot (Figure 4-5A).

4.5 Conclusion

Pawpaw fruit remains underutilized as a specialty crop, with very little published data on the properties of the cultivated pawpaw fruit and even less data on established objective indicators of ripeness. The data from this study show that unripe and ripe pawpaw fruits have significantly different skin and pulp colors. Unripe pawpaw fruits are much harder and have a higher chewiness than ripe pawpaw fruits. Due to the conversion of complex sugars to simpler sugars that occurs during ripening, the ripe pawpaw fruits have a lower cohesiveness than unripe fruits. As a result, it may not be suitable for ripe pawpaw fruits to be packed on top of each other during transportation. From the correlation analysis, it was found that the skin a* values and hue angles have a strong correlation with the textural properties of the fruits, hence, the skin a* values and hue angles can be used as noninvasive indices of pawpaw ripeness. Potentially, farmers can use commercially available handheld color meter and Brix meters to determine pawpaw fruit ripeness noninvasively based on specific skin color indices. This study was conducted on wild fruit from one location and results may be different for specific cultivars of pawpaw grown in

different environments. The findings from this study establish a foundation for further research on better indicators of pawpaw fruit ripeness solutions to prevent pawpaw fruit loss.

Brief Synopsis: Chapter 5 contains an experiment on the effect of chitosan and sodium alginate edible coatings and freshness paper treatments on the quality of different cultivars of the North American pawpaw fruits during cold storage. The contents of this chapter have been published in the *Current Research in Food Science* journal.

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Chapter 5

A comparative study of edible coatings and freshness paper on the quality of fresh North American pawpaw (*Asimina triloba*) fruits using TOPSIS-Shannon entropy analyses

Abstract

The North American pawpaw (Asimina triloba) is a tropical fruit that is known to be the largest edible fruit native to the United States. The fruit has remained uncommercialized because of the rapid changes in quality that occur after the fruit is harvested. However, only a few studies have evaluated the quality of the fruit during postharvest storage. This study aimed to assess the effect of different concentrations of chitosan and sodium alginate coatings, and freshness paper treatments on the quality characteristics of pawpaw fruits during storage and use TOPSIS-Shannon entropy analyses to determine which treatment best maintains the quality of the fruits from three cultivars. The results show that the chitosan coatings were more effective in slowing moisture loss in Sunflower fruits than in Susquehanna and 10-35 fruits over time. Similarly, the freshness paper treatment controlled moisture loss more effectively than sodium alginate coatings. The 10-35 fruits with 1% chitosan coating had very little change in skin color and physical appearance compared to all the other treatments. The TOPSIS-Shannon entropy analyses showed that the 10-35 fruits with 1% chitosan had the most stable quality over time, followed by the Susquehanna and Sunflower fruits with 2% chitosan coatings. The experimental data from different cultivars, treatments, and storage conditions, proved the shelf-life of pawpaw fruit could be extended from 5 days to 15-20 days depending on the

cultivar. These findings will enable the creation of markets for pawpaw fruits and allow countries that grow them to generate revenue from this underutilized specialty crop.

5.1 Introduction

The North American pawpaw (*Asimina triloba*) is the largest edible fruit native to the United States; however, the fruit has remained underutilized due to the rapid change in quality during storage. It grows in over 30 states in the United States, Canada, and other parts of the world. The fruit belongs in the Annonaceae family with many commercially produced tropical fruits like soursop, cherimoya, sugar apple, and others. Pawpaw is a low acid fruit that has a very short shelf life characterized by rapid discoloration of the skin and pulp, and loss in fruit firmness within 5 days (Adainoo et al., 2022; Galli et al., 2008). Some studies have attempted to extend the shelf life of the fruit with the application of cold storage technologies. However, these have had limited success in retaining the quality characteristics of the whole fruit during the storage period. To date, no studies have been conducted to test the effect of edible coatings and freshness paper treatments on the quality characteristics of the North American pawpaw fruit during storage, making this the first research attempt at extending the shelf life of the whole pawpaw fruit using edible coatings and freshness paper treatments. Studying the effect of technologies that could extend the shelf life of the fruit would directly promote the creation of a market niche for shelf-stable pawpaw fruits thereby driving the economic growth of the fruit in countries that cultivate it. Studies on other fruits in the Annonaceae family like cherimoya and soursop have shown that edible coatings help to maintain postharvest quality and extend the shelf life (de Los Santos-Santos et al., 2020; Liu et al., 2016).

Edible coatings are environmentally friendly systems that when applied to horticultural products control moisture loss and gas transfer during the respiration of fruits and vegetables after postharvest, thereby controlling their quality characteristics and extending the shelf life of the products (Dhall, 2013; Souza et al., 2010). Although some fruits and vegetables respire more than others, generally, they all continue to respire after they have been harvested. Hence, these edible coating systems provide storage conditions similar to modified atmosphere storage systems that help to preserve the fruits and vegetables by controlling the internal gas composition of the fruit or vegetable (Park, 1999). Edible coatings have been successfully used to extend the shelf lives of whole fruits and vegetables including apples, oranges, peaches, lemons, avocados, and tomatoes. The coatings have also been successfully applied to fresh-cut fruits and vegetables, nuts, seeds, cheese, and other food products (Chiumarelli et al., 2011; Zambrano-Zaragoza et al., 2018). There are several advantages of edible coatings. They are economical, readily available, offer barrier properties and some mechanical strength, and help to prevent contamination of the fruit skin, which reduces the chances of fruit deterioration by preventing microbial contamination, browning, development of off-flavors, solute migration, and texture breakdown (Dhall, 2013; Zambrano-Zaragoza et al., 2018).

Edible coatings can be made of polysaccharides, proteins, lipids, or a blend of these components. Examples of materials that have been used in edible coatings include sodium alginate, methylcellulose, chitosan, pectin, aloe vera gel, whey proteins, soy proteins, and lactic acid (Yousuf et al., 2018). Chitosan coatings have been known to possess antimicrobial properties in addition to the barrier properties they offer. Further, edible coating formulations can be modified with the addition of other preservation agents like

essential oils, organic acids, polypeptides, nanoemulsions, nanotubes, nanoparticles, and other nanosystems to improve the efficiency of the coating system (Dhall, 2013; Franssen & Krochta, 2003; Zambrano-Zaragoza et al., 2018).

Various studies have explored the potential of loaded paper technologies (paper loaded with essential oil or antimicrobial compounds) for extending the shelf life of foods. These technologies provide the opportunity for creating an active packaging that continually releases preserving agents like essential oils to prevent superficial microbial growth, extend shelf life and in some cases enhance the sensory appeal of foods (Ataei et al., 2020; Shao et al., 2021). In this study, the freshness paper used is a commercially available fenugreek-loaded paper for preserving perishable products (Shukla, 2002).

The aim of this study was to evaluate the effect of different concentrations of chitosan and sodium alginate coatings, and freshness paper treatments on the quality characteristics of three cultivars of North American pawpaw fruits and use TOPSIS-Shannon entropy analyses to compare the treatments to identify which one best maintains the quality of the fruits from three cultivars during storage.

5.2 Materials and Methods

5.2.1 Fruit Samples

Ripe pawpaw fruits harvested from the lower orchard at the Southwest Research, Extension, and Education Center of the University of Missouri (lat. 37.08582, long. −93.86713) were used for this study. The orchard had a fertile alluvial soil that was deep and well-drained. Seventy-three ripe pawpaw fruits of different cultivars (Susquehanna,

Sunflower, Shenandoah, Atwood, 10-35, Wells, Wilson, Prolific, NC-1) were harvested at peak ripeness in September 2021, placed in zippered plastic bags and transported to the laboratory on ice. The fruits were harvested at peak ripeness, which was determined by the pitting on the skin when the fruit is gently pressed with a finger. These fruits were mixed and treated with freshness papers and sodium alginate coatings and studied over a 25-day storage period.

Ripe pawpaw fruits of the Susquehanna (71 fruits), Sunflower (101 fruits) and 10-35 cultivars (99 fruits) were harvested from the same orchard at peak ripeness in August/September 2022, placed in open totes and transported to the laboratory. These fruits were treated separately by cultivar with chitosan coatings and studied over a 25-day storage period.

5.2.2 Treatments

From the mixed fruit group, fruits of similar color and size were selected and randomly divided into four groups for the treatments: control, freshness paper treatment (FP), coating with 1g sodium alginate in 1L distilled water (0.001% alginate) and coating with 5g sodium alginate in 1L distilled water (0.005% alginate). Control fruits received no treatment. The control fruits were placed in an open Styrofoam box. Fruits given freshness paper treatment were placed in an open Styrofoam box, and freshness papers (Freshpaper, The Freshglow Co., Maryland, USA) cut into 5×4cm pieces were placed on top of each fruit in the box. The sodium alginate treated fruits were also placed in separate open Styrofoam boxes. All the boxes with the fruits were kept in a refrigerator at 4°C (75% RH)

and 3-5 fruits randomly selected from each treatment were analyzed at 5-day intervals for pH, titratable acidity, total soluble solids, moisture loss, skin color, hardness, and cohesiveness ratio.

The fruits for the each of the cultivars (Susquehanna, Sunflower and 10-35) were each randomly grouped into three subsets for treatments: control, 1% chitosan coating and 2% chitosan coating. The fruits for each cultivar and treatment were placed in separate open totes and stored in a cold room with an average temperature of 6ºC (80% RH), and 3-5 fruits randomly selected from each cultivar-treatment combination were analyzed at 5-day intervals for pH, titratable acidity, total soluble solids, moisture loss, skin color, hardness, and cohesiveness ratio.

5.2.2.1 Preparation of sodium alginate coating solutions and coating of pawpaw fruits

Two different concentrations of sodium alginate were used in this study. 0.001% (w/v) and 0.005% (w/v) sodium alginate solution were prepared by dissolving sodium alginate (MP Biomedicals, Solon, OH, USA) in distilled water while stirring. A 2% (w/v) solution of calcium chloride (Fisher Scientific, NJ, USA) was prepared to be used in the coating to induce crosslinking of the sodium alginate for the formation of the coating film on the skin of the fruits.

The fruits were washed with tap water at room temperature to remove debris on the skin. The fruits were coated following the method outlined by Maftoonazad et al. (2008). Pawpaw fruits for the two treatments: 0.001% sodium alginate coating and 0.005% sodium alginate coating, were dipped in the respective sodium alginate coating solutions for 60

seconds at 20°C and the excess coating solution was allowed to drip off. The fruits were then immersed in the calcium chloride solution for 30 seconds. The films formed on the fruits were dried by blowing air on the surface of the fruits with a tabletop fan.

5.2.2.2 Preparation of chitosan coating solutions and coating of pawpaw fruits

The chitosan coating solutions were prepared according to the procedure described by Arnon et al. (2015). 1% (w/v) and 2% (w/v) chitosan coating solutions were prepared and used in this study. Chitosan solution was prepared by dissolving low molecular weight (50,000-190,000 Da) chitosan powder (Sigma Aldrich, St. Louis, MO) in distilled water containing 0.07% (v/v) glacial acetic acid (Fisher Chemicals, Fair Lawn, NJ). The chitosan solutions were stirred at room temperature with a magnetic stirrer overnight. The pH of the chitosan solutions was adjusted with 0.1N sodium hydroxide to a pH of 5.01.

The fruits were washed with tap water at room temperature to remove debris on the skin and sanitized by wiping the skin with paper tissue containing 70% ethanol. The fruits were then coated by dipping them in the chitosan solution for 60 seconds. They were then removed from the chitosan solution and placed on a rack to allow the excess coating solutions to drip off and dried by blowing air on the surface of the fruits with a tabletop fan.

5.2.3 pH and Titratable acidity

The pH of the pulp was measured using a digital pH meter (SevenCompact S220, Mettler Toledo, Greifensee, Switzerland) at room temperature (25ºC). The measurements

were taken in triplicates. Titratable acidity was determined according to the AOAC Official Method 942.15 (AOAC, 2000). Five grams of the fruit pulp was mixed with 25ml of distilled water, blended in a kitchen blender for 2 minutes to obtain a homogeneous mixture and titrated against 0.1N NaOH using phenolphthalein as indicator. The analyses were performed in triplicates and the titratable acidity was reported as milligrams of acetic acid per 100ml of sample (Nam et al., 2018).

$$\text{Titratable acidity} = \frac{\text{NaOH normality} \times \text{Titre value} \times \text{Acetic acid eq. weight} \times 100}{\text{Sample weight} \times 1000}$$

Acetic acid eq. weight = 60.052 g

5.2.4 Total soluble solids

Total soluble solids (TSS) content was measured according to the AOAC Official Method 932.14C (AOAC, 2000) using a digital refractometer (HI96800, Hanna Instruments, Woonsocket, RI, USA) at room temperature (25ºC). A sample of the pulp was placed in the sample well of the refractometer. The total soluble solids measurements were taken in triplicates and recorded as Brix.

5.2.5 Percentage moisture loss

Moisture loss was determined by weighing the fruits at 5-day intervals (final weight) using a digital balance and reporting the difference in weight compared to their weight on day 0 (initial weight) as percentage moisture loss.

$$\%\ \text{moisture loss} = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \times 100$$

5.2.6 Skin color

Color of fruit skin was measured using the Hunter LAB color meter (Chroma Meter CR-410, Konica Minolta, Tokyo, Japan). Four readings for each fruit were read at four different points on the skin of the fruit. The recordings were used to calculate the total color difference (ΔE or Delta E), and chroma using the equations below where, L* is degree of lightness to darkness, a* is degree of redness to greenness, b* is degree of yellowness to blueness; the subscripts *f* and *i* denote final (day 5-25 fruits) and initial (day 0 fruits) values and presented as means and standard deviations. Chroma represents the saturation of the skin color and ΔE is a measure of total color difference between a reference color (skin color at day 0) and the skin color at a particular time point (skin color at days 5-25).

$$\Delta E = \sqrt{(L*_f - L*_i)^2 + (a*_f - a*_i)^2 + (b*_f - b*_i)^2}$$

$$Chroma = \sqrt{a*^2 + b*^2}$$

5.2.7 Texture analyses

Textural properties of the fruits were determined following the method described by Adainoo et al. (2023). Samples were analyzed for their hardness and cohesiveness using a Texture Analyzer (TA.HDPlus C, Stable Micro Systems, Surrey, UK) equipped with a 100kg load cell and connected to the Exponent Connect Software (Stable Micro Systems). A P/75 (7.5cm diameter) compression plate was used for the analyses. The texture analyzer was programmed to carry out a texture profile analysis with the following test conditions: pretest speed of 1mm/sec, test speed of 1mm/sec, posttest speed of 1mm/sec, trigger force of 5g, compression distance of 10mm and a time of 5sec between compressions. Hardness was recorded as kilograms of force (kg) and cohesiveness ratio was recorded as a

dimensionless ratio. Hardness values were the maximum force of the first compression in a Texture Profile Analysis (TPA) while cohesiveness is a measure of the strength of the internal bonds that keep a food sample intact (Kamal-Eldin et al., 2020; Kasapis & Bannikova, 2017). Cohesiveness, in a TPA, is ratio of the area of second compression to the area of first compression as expressed in the equation below. Three to five fruits for each treatment-time combination were tested and the results were averaged.

$$\text{Cohesiveness ratio} = \frac{\text{Area 2}}{\text{Area 1}} \times 100$$

5.2.8 TOPSIS-Shannon entropy analyses

In this study, TOPSIS-Shannon entropy analyses were used to decide which treatment and cultivar performed best in extending the shelf life of the pawpaw fruits based on the physical and physicochemical properties analyzed. The Shannon entropy method was used to determine the weight vectors for the criteria (the parameters analyzed) which was then used in the TOPSIS (technique for order preference by similarity to ideal solution) analyses. TOPSIS was carried out with the data obtained to evaluate the effect of the different treatments on the fruits from the different cultivars. From this, the distance of alternatives (the different treatments on the cultivars) from the positive and negative ideal solutions were determined and the treatments were ranked. The analyses were carried out as described by Ansarifar et al. (2015) and Khodaei et al., (2021). The method is summarized as follows:

1. Construction of a decision-making matrix with a list of alternatives (treatments) as row labels and the factors to be considered (physical and physicochemical

properties analyzed) as column headings using the mean for each of the factors over the storage period

2. Normalization of the decision-making matrix

$$r_{ij} = \frac{X_{ij}}{\sqrt{\sum X_{ij}^2}}$$

i=1, 2……m, j=1, 2……n; m = number of alternatives, n = number of factors considered

3. Calculation of the weights for the criterion and develop the normalized weight matrix

$$V_{ij} = W_{ij} \times r_{ij}$$

The weight of each criterion was determined using the Shannon entropy method using the following steps:

a. Design the decision-making matrix

b. Design the normalized decision-making matrix

$$P_{ij} = \frac{X_{ij}}{\sum_{i=1}^{m} X_{ij}},\ j = 1 \ldots n$$

c. Calculation of the entropy for each criterion

$$E_j = -h \sum_{i=1}^{m} \left(P_{ij} \times \ln P_{ij}\right), \qquad h = \frac{1}{\ln(m)}, 0 \leq E_j \leq 1$$

d. Calculation of the distance of each criterion from the entropy (degree of diversification)

$$d_j = 1 - E_j$$

e. Calculation of the weights of each criterion from the entropy

$$W_j = \frac{d_j}{\sum_{k=1}^{n} d_j}$$

4. Determination of the ideal solution: the ideal best solution is made of the optimal value of every factor from the weighted decision-making matrix, and the ideal worst solution is made of the worst value of every factor from the weighted decision-making matrix.

$$V_i^+ = (V_1^+, V_2^+, V_3^+, \dots, V_m^+)$$

$$V_i^- = (V_1^-, V_2^-, V_3^-, \dots, V_m^-)$$

Where the ideal value and negative ideal value are determined by how the maximum and minimum values of the factors affect the quality of the fruit. For example, high L* values suggest more fresh ripe fruits compared to ripe fruits with low L* values. Hence, a high weighted performance value will be the ideal best for L* and a low weighted performance value will be the ideal worst for L*.

5. Determination of the distance of the normalized weighted matrix from ideal best and ideal worst (Euclidean distances)

$$S_i^+ = \sqrt{\sum \left(V_{ij} - V_i^+\right)^2}$$

$$S_i^- = \sqrt{\sum \left(V_{ij} - V_i^-\right)^2}$$

6. Calculation of the performance scores

$$P_i = \frac{S_i^-}{S_i^+ + S_i^-}$$

7. Ranking the treatments

5.2.9 Statistical analyses

All experimental data are presented as mean ± standard deviation. The data for the treatments at the time intervals (Day 0, 5, 10, 15, 20 and 25) were analyzed by analysis of variance (ANOVA) and Tukey's test ($p<0.05$) for significant differences using JMP 14.0.0 software (SAS Institute Inc., Cary, USA). The overall variability (in terms of the parameters analyzed) among treatments during storage was analyzed using Principal Component Analysis (PCA). PCA was performed using OriginPro 2021 version 9.8.0 software (Origin Lab Inc., Northampton, Massachusetts, USA). Microsoft Excel version 16.69 was used to run the TOPSIS analyses.

5.3.0 Results and Discussion

5.3.1 pH and titratable acidity

Fruit acidity is a key indicator of quality because organic acids present in fruits contribute significantly to their flavor and aroma volatiles (Batista-Silva et al., 2018). In fruits like strawberries and mangoes, it has been found that during storage, pH increases while titratable acidity decreases possibly due to the oxidation of the acids during storage (Alharaty & Ramaswamy, 2020; Cosme Silva et al., 2017; Islam et al., 2013). Other studies have reported that for fruits in the Annonaceae family like atemoya and soursop, and others like banana, pH decreases, and titratable acidity increases during storage (Pareek et al., 2011; Rahman et al., 2013; Torres et al., 2009). This is likely due to the production of more organic acids from the fermentation of the sugars in the fruits.

The data obtained in this study show that Susquehanna fruits in the control group and the chitosan treatment groups had no significant change ($p>0.05$) in their pH and titratable acidity during the storage period at 4ºC (Table 5-1). However, there was a higher difference in the pH of the control fruits between day 0 (6.23±0.49) and day 15 (5.65±0.54) compared to the difference in pH for the 2% chitosan coated fruits between day 0 (6.36±0.47) and day 15 (6.00±0.81).

The pH difference between day 0 and day 15 for both control and 1% chitosan coated fruits was similar. Further, it took up to day 20 for the pH of the 2% chitosan coated fruits to reach a pH less than 5.98, which is the minimum pH for good quality Susquehanna pawpaw pulp according to Adainoo et al. (2022), whereas the 1% chitosan coated fruits recorded an average pH less than 5.98 by day 10. This suggests that the 2% chitosan coating was more effective in controlling the change in pH of the Susquehanna fruits during storage. For all the Susquehanna fruit treatments, there were no clear patterns in the change of the titratable acidity of the fruits during the storage period. This may have been due to the change in the type of acid and concentration of the different acids during the storage since studies have shown that organic acid composition and concentrations in pawpaw fruits change as they continue to mature (Park et al., 2022). The pulp of unripe fruits has a high concentration of citric acid (229.98±2.19 mg/100g fresh weight) and no acetic acid detected, but as they ripen and mature, the concentration of citric acid reduces to an average of 8.65-16.20 mg/100g fresh weight and acetic acid becomes the predominant acid with a concentration of 61.59±0.92 mg/100g fresh weight (Pande & Akoh, 2010; Park et al., 2022). Further, the titratable acidity of the Susquehanna fruits used in this study were

significantly higher than the values (45.00±15.97 mg of acetic acid/100g) reported by Adainoo et al. (2022).

Table 5-1: Physicochemical properties, skin color, and textural properties of chitosan-coated North American pawpaw (*Asimina triloba*) fruits of the Susquehanna cultivar during a 25-day storage at 6ºC

Treatment	Day	pH	Titratable Acidity (mg of acetic acid/100ml)	Total soluble solids (Brix)	Moisture loss (%)	L*
Control	0	6.23±0.49 ^{aA}	101.42±16.67 ^{aA}	24.09±2.82 ^{aA}	0.00±0.00 ^{bF}	45.75±1.43 ^{aA}
	5	5.84±0.61 ^{aA}	168.15±40.03 ^{aA}	24.94±5.77 ^{aA}	7.20±0.52 ^{aAB}	41.69±1.52 ^{bA}
	10	5.79±0.78 ^{aA}	130.78±30.31 ^{aA}	24.73±2.98 ^{aA}	7.20±0.52 ^{aAB}	35.99±1.47 ^{cA}
	15	5.65±0.54 ^{aA}	136.12±42.37 ^{aA}	18.94±7.21 ^{aA}	7.62±0.25 ^{aA}	32.42±0.73 ^{dA}
1% Chitosan	0	6.20±0.69 ^{aA}	106.76±12.23 ^{aA}	23.64±1.16 ^{aA}	0.00±0.00 ^{dF}	50.92±3.94 ^{aA}
	5	6.06±0.13 ^{aA}	112.10±13.87 ^{aA}	22.96±1.52 ^{aA}	3.78±0.70 ^{cE}	44.16±10.04 ^{aA}
	10	5.61±0.30 ^{aA}	117.44±20.15 ^{aA}	24.09±3.82 ^{aA}	4.45±0.30 ^{bcCDE}	37.60±10.47 ^{aA}
	15	5.56±0.32 ^{aA}	114.77±12.23 ^{aA}	22.29±3.00 ^{aA}	5.61±0.51 ^{abBCD}	36.83±9.56 ^{aA}
	20	5.75±0.74 ^{aA}	98.75±32.36 ^{aA}	22.04±0.69 ^{aA}	5.76±0.26 ^{abBCD}	35.08±9.93 ^{aA}
	25	5.57±0.61 ^{aA}	114.77±25.74 ^{aA}	22.88±1.81 ^{aA}	6.94±0.86 ^{aAB}	35.30±10.03 ^{aA}
2% Chitosan	0	6.36±0.47 ^{aA}	122.77±25.74 ^{aA}	26.29±2.72 ^{aA}	0.00±0.00 ^{cF}	50.89±5.15 ^{aA}
	5	6.26±0.60 ^{aA}	104.09±0.00 ^{aA}	18.17±2.89 ^{bA}	4.12±1.09 ^{bDE}	51.20±2.89 ^{aA}
	10	6.28±0.67 ^{aA}	109.43±23.11 ^{aA}	23.44±0.52 ^{abA}	4.27±0.13 ^{bCDE}	48.29±5.50 ^{aA}
	15	6.00±0.81 ^{aA}	101.42±64.72 ^{aA}	23.08±2.24 ^{abA}	5.61±0.94 ^{abBCD}	47.15±6.66 ^{aA}
	20	5.72±0.46 ^{aA}	88.08±36.69 ^{aA}	24.84±3.93 ^{abA}	6.01±0.63 ^{abABC}	42.10±9.83 ^{aA}
	25	5.34±0.11 ^{aA}	165.48±28.12 ^{aA}	24.21±2.03 ^{abA}	6.86±0.79 ^{aAB}	39.99±9.69 ^{aA}

Control fruits showed the presence of mold growth on the fruit skin after day 15, hence, they were not analyzed after day 15.
Means for the parameters in each treatment that do not share a superscript are significantly different at $p \leq 0.05$.
Lowercase superscripts represent statistical differences within treatment groups for the respective treatments and uppercase superscripts represent statistical differences between all treatment groups at $p \leq 0.05$.

Table 5-1: Physicochemical properties, skin color, and textural properties of chitosan-coated North American pawpaw (*Asimina triloba*) fruits of the Susquehanna cultivar during a 25-day storage at 6ºC (continued)

Treatment	Day	a*	b*	Chroma	ΔE	Hardness (kg)	Cohesiveness ratio (%)
Control	0	-9.56±0.44 ^{cA}	19.86±1.06 ^{aA}	22.07±0.79 ^{aA}	0.00±0.00 ^{dB}	5.4±0.7 ^{aA}	29.76±2.96 ^{bD}
	5	-5.71±1.07 ^{bA}	17.43±2.27 ^{aA}	18.41±2.11 ^{bA}	7.03±1.42 ^{cAB}	3.8±0.8 ^{abAB}	31.98±2.16 ^{bCD}
	10	-1.15±2.00 ^{aA}	10.99±0.40 ^{bA}	11.23±0.48 ^{cA}	15.73±3.06 ^{bAB}	2.5±1.4 ^{bB}	44.31±3.66 ^{aABCD}
	15	1.34±1.08 ^{aA}	6.22±1.18 ^{cA}	6.56±1.23 ^{dA}	22.10±1.19 ^{aAB}	2.4±0.6 ^{bB}	44.56±6.04 ^{aABC}
1% Chitosan	0	-8.00±5.35 ^{aA}	24.90±4.02 ^{aA}	26.55±5.10 ^{aA}	0.00±0.00 ^{aB}	4.5±1.3 ^{aAB}	34.56±7.72 ^{bCD}
	5	-4.68±7.87 ^{aA}	19.68±10.04^{aA}	21.19±10.65^{aA}	11.12±8.46 ^{aAB}	4.2±0.4 ^{aAB}	35.86±4.01 ^{bBCD}
	10	-1.84±7.11 ^{aA}	13.60±11.39^{aA}	15.05±11.41^{aA}	19.48±11.30 ^{aAB}	3.1±1.2 ^{aAB}	37.37±5.48 ^{bABCD}
	15	-0.38±7.00 ^{aA}	11.68±10.02^{aA}	13.20±10.05^{aA}	21.88±10.53 ^{aAB}	2.7±1.0 ^{aB}	44.06±5.27 ^{abABCD}
	20	0.00±6.22 ^{aA}	8.28±10.12 ^{aA}	10.11±9.94 ^{aA}	24.59±13.53 ^{aA}	2.4±0.9 ^{aB}	51.80±0.65 ^{aA}
	25	-0.19±6.24 ^{aA}	8.31±10.89 ^{aA}	10.17±10.68^{aA}	25.00±13.21 ^{aA}	2.0±0.2 ^{aB}	51.74±0.89 ^{aA}
2% Chitosan	0	-9.95±5.19 ^{aA}	23.34±3.55 ^{aA}	26.16±4.33 ^{aA}	0.00±0.00 ^{bB}	4.2±0.3 ^{aAB}	32.62±4.73 ^{bCD}
	5	-11.70±1.95 ^{aA}	24.33±2.17 ^{aA}	27.02±2.79 ^{aA}	5.54±2.90 ^{abAB}	4.1±0.8 ^{aAB}	34.61±3.43 ^{bCD}
	10	-9.56±3.39 ^{aA}	20.93±4.65 ^{aA}	23.07±5.60 ^{aA}	9.18±2.26 ^{abAB}	3.6±1.5 ^{abAB}	34.44±10.33 ^{bCD}
	15	-7.88±4.00 ^{aA}	19.70±5.62 ^{aA}	21.30±6.69 ^{aA}	9.06±3.22 ^{abAB}	2.7±0.1 ^{abB}	43.60±3.96 ^{abABCD}
	20	-4.42±6.55 ^{aA}	14.07±9.11 ^{aA}	15.09±10.62 ^{aA}	15.58±8.52 ^{aAB}	1.9±0.2 ^{abB}	50.22±4.10 ^{abAB}
	25	-2.93±7.02 ^{aA}	11.86±10.37 ^{aA}	13.01±11.42 ^{aA}	18.85±9.64 ^{aAB}	2.6±0.6 ^{bB}	42.42±1.68 ^{aABCD}

Control fruits showed the presence of mold growth on the fruit skin after day 15, hence, they were not analyzed after day 15.
Means for the parameters in each treatment that do not share a superscript are significantly different at $p \leq 0.05$.
Lowercase superscripts represent statistical differences within treatment groups for the respective treatments and uppercase superscripts represent statistical differences between all treatment groups at $p \leq 0.05$.

The Sunflower control fruits had no statistically different pH from day 0 to day 10, but at day 15 the pH of the fruits was significantly different from the pH at the previous time points (Table 2). The pH of the Sunflower fruits with the 1% chitosan coating on the other hand had no significant difference during the storage period until day 25, whereas the Sunflower fruits with the 2% chitosan coating had no significant difference in pH throughout the 25-day storage period. This shows that both chitosan coatings were effective in controlling the change in pH of the Sunflower fruits during storage. The titratable acidity of the control fruits increased with time suggesting the fermentation of the sugars in the fruits, however, in the chitosan coated fruits, the titratable acidity remained statistically similar throughout the storage period and close to the values reported for Sunflower fruits by Adainoo et al. (2022). This shows the chitosan coatings were effective in controlling the formation of acids in the Sunflower fruits during storage.

Table 5-2: Physicochemical properties, skin color, and textural properties of chitosan-coated North American pawpaw (*Asimina triloba*) fruits of the Sunflower cultivar during a 25-day storage at 6ºC

Treatment	Day	pH	Titratable Acidity (mg of acetic acid/100ml)	Total soluble solids (Brix)	Moisture loss (%)	L*
Control	0	6.53±0.22 ^{aABC}	85.41±4.62 ^{bABCD}	18.49±1.05 ^{Aa}	0.00±0.00 ^{cF}	45.65±0.98 ^{aABCDE}
	5	6.43±0.04 ^{aABC}	106.76±16.67 ^{abAB}	19.40±0.46 ^{aA}	5.04±0.74 ^{bBC}	35.28±3.74 ^{bDEF}
	10	6.28±0.04 ^{aABCD}	93.41±16.67 ^{abABC}	19.07±1.11 ^{aA}	6.01±0.88 ^{abAb}	31.13±1.23 ^{bcEF}
	15	5.67±0.20 ^{bD}	122.77±4.62 ^{aA}	19.48±1.17 ^{aA}	7.39±1.35 ^{aA}	27.42±0.61 ^{cF}
1% Chitosan	0	6.71±0.05 ^{aAB}	80.07±24.02 ^{aBCDE}	20.09±0.47 ^{aA}	0.00±0.00 ^{dF}	60.04±0.53 ^{aA}
	5	6.64±0.09 ^{aAB}	61.39±20.15 ^{aCDEF}	19.52±3.73 ^{aA}	1.64±0.41 ^{cEF}	54.63±2.80 ^{aABC}
	10	6.49±0.13 ^{aABC}	48.04±8.01 ^{aDEF}	18.63±0.50 ^{aA}	1.64±0.41 ^{cEF}	50.73±5.55 ^{aABCD}
	15	6.70±0.19 ^{aAB}	40.03±13.87 ^{aF}	20.18±1.87 ^{aA}	2.58±0.45 ^{bDE}	47.93±8.91 ^{aABCDE}
	20	6.54±0.35 ^{aABC}	58.72±12.23 ^{aCDEF}	18.40±1.61 ^{aA}	2.81±0.17 ^{bDE}	41.93±13.39 ^{aBCDEF}
	25	5.94±0.07 ^{bCD}	50.71±4.62 ^{aDEF}	19.78±0.53 ^{aA}	3.97±0.23 ^{aCD}	39.84±12.35 ^{aCDEF}
2% Chitosan	0	6.78±0.21 ^{abA}	53.38±12.23 ^{aDEF}	19.40±1.21 ^{aA}	0.00±0.00 ^{cF}	58.24±1.84 ^{aAB}
	5	6.66±0.43 ^{abAB}	42.70±12.23 ^{aEF}	19.81±1.34 ^{aA}	1.43±0.37 ^{bEF}	55.66±1.50 ^{aABC}
	10	6.56±0.23 ^{abABC}	42.70±9.25 ^{aEF}	18.66±1.06 ^{aA}	1.43±0.37 ^{bEF}	54.05±0.46 ^{abABC}
	15	6.89±0.22 ^{aA}	32.03±13.87 ^{aF}	18.91±2.97 ^{aA}	2.57±0.42 ^{abDE}	50.49±2.78 ^{abABCD}
	20	6.40±0.39 ^{abABC}	37.37±4.62 ^{aF}	18.71±0.82 ^{aA}	3.70±0.62 ^{aCD}	45.21±6.06 ^{bcABCDE}
	25	6.08±0.06 ^{bBCD}	48.04±0.20 ^{aDEF}	18.74±0.91 ^{aA}	3.70±0.62 ^{aCD}	39.51±4.76 ^{cCDEF}

Control fruits showed the presence of mold growth on the fruit skin after day 15, hence, they were not analyzed after day 15.
Means for the parameters in each treatment that do not share a superscript are significantly different at $p \leq 0.05$.
Lowercase superscripts represent statistical differences within treatment groups for the respective treatments and uppercase superscripts represent statistical differences between all treatment groups at $p \leq 0.05$.

Table 5-2: Physicochemical properties, skin color, and textural properties of chitosan-coated North American pawpaw (*Asimina triloba*) fruits of the Sunflower cultivar during a 25-day storage at 6ºC (continued)

Treatment	Day	a*	b*	Chroma	ΔE	Hardness (kg)	Cohesiveness ratio (%)
Control	0	-7.26±1.55 ^{bAB}	24.09±0.59 ^{aABCD}	25.36±0.90 ^{aABCDE}	0.00±0.00 ^{cC}	4.2±0.4 ^{abABC}	24.90±2.02 ^{aEF}
	5	0.76±1.37 ^{aAB}	12.11±5.43 ^{bDEF}	12.59±4.92 ^{bDEF}	18.08±5.79 ^{bABC}	4.6±0.4 ^{aA}	21.99±1.87 ^{aF}
	10	2.91±0.46 ^{aA}	5.65±0.12 ^{bcEF}	6.89±0.06 ^{bcEF}	25.79±0.81 ^{abAB}	3.5±0.3 ^{bcABCDEF}	27.83±3.13 ^{aDEF}
	15	2.99±0.25 ^{aA}	0.91±0.93 ^{cF}	3.48±0.64 ^{cF}	31.36±2.00 ^{aA}	2.8±0.4 ^{cDEFG}	27.68±3.26 ^{aDEF}
1% Chitosan	0	-8.65±1.25 ^{Aab}	37.77±3.42 ^{aA}	39.52±2.67 ^{aA}	0.00±0.00 ^{bC}	4.3±0.3 ^{aAB}	32.22±4.39 ^{bABCDEF}
	5	-7.26±6.24 ^{aAB}	34.27±2.37 ^{aAB}	35.47±2.51 ^{aAB}	11.42±0.71 ^{abABC}	3.8±0.9 ^{abABCD}	31.32±5.01 ^{bBCDEF}
	10	-5.14±7.85 ^{aAB}	29.80±4.05 ^{aABCD}	30.91±5.26 ^{aABCD}	16.36±3.53 ^{abABC}	3.3±0.4 ^{abcBCDEF}	31.97±2.24 ^{bABCDEF}
	15	-3.79±8.25 ^{aAB}	25.06±7.89 ^{aABCD}	26.20±9.11 ^{aABCD}	22.19±8.30 ^{abABC}	3.0±0.5 ^{abcCDEF}	40.10±4.47 ^{abAB}
	20	-1.13±9.21 ^{aAB}	19.14±13.57 ^{aBCDEF}	20.54±14.11 ^{aBCDEF}	30.25±16.62 ^{abAB}	2.4±0.5 ^{bcEFG}	42.34±1.11 ^{abA}
	25	0.64±6.55 ^{aAB}	14.94±13.23 ^{aCDEF}	16.13±12.99 ^{aCDEF}	33.75±17.11 ^{aA}	2.3±0.1 ^{cFG}	38.27±2.60 ^{aABC}
2% Chitosan	0	-12.16±1.36 ^{bB}	35.37±3.20 ^{aAB}	37.50±2.63 ^{aAB}	0.00±0.00 ^{cC}	4.1±0.2 ^{aABC}	28.83±1.65 ^{bCDEF}
	5	-10.15±3.43 ^{abAB}	34.41±3.42 ^{aAB}	36.11±2.57 ^{aAB}	6.81±1.76 ^{bcBC}	3.7±0.5 ^{abABCDE}	27.05±1.95 ^{bDEF}
	10	-6.31±4.01 ^{abAB}	32.68±1.53 ^{abABC}	34.04±0.74 ^{aABC}	9.92±4.71 ^{bcABC}	2.6±0.2 ^{bcDEFG}	34.92±0.66 ^{abABCDE}
	15	-6.09±4.97 ^{abAB}	28.91±1.61 ^{abABCD}	29.92±2.40 ^{abABCD}	13.21±7.43 ^{abcABC}	2.8±0.6 ^{cdDEFG}	40.95±6.12 ^{aAB}
	20	-2.85±4.85 ^{abAB}	21.99±7.42 ^{bcABCDE}	22.70±7.74 ^{bcABCDE}	21.35±12.77 ^{abABC}	2.2±0.1 ^{cdFG}	35.47±5.72 ^{abABCD}
	25	0.08±3.69 ^{aAB}	13.77±5.17 ^{cDEF}	14.80±4.64 ^{cDEF}	31.25±10.27 ^{aA}	1.7±0.1 ^{dG}	36.33±2.79 ^{abABCD}

Control fruits showed the presence of mold growth on the fruit skin after day 15, hence, they were not analyzed after day 15.
Means for the parameters in each treatment that do not share a superscript are significantly different at $p \leq 0.05$.
Lowercase superscripts represent statistical differences within treatment groups for the respective treatments and uppercase superscripts represent statistical differences between all treatment groups at $p \leq 0.05$.

All the fruits of the 10-35 cultivar had no visible mold growth on their skin throughout the study, hence were analyzed for the full duration. During this period, there were slight changes in the pH of the fruit pulp of the 10-35 control and 1% chitosan coated fruits, but the pH of the 2% chitosan coated fruits remained statistically similar (Table 5-3). Nonetheless, the titratable acidity remained statistically similar for all the 10-35 treatment groups. The reason for this is in the control fruits unclear, however, this may be attributed to the changes in the composition and concentrations of the organic acids in the fruits of this cultivar over time as identified by Park et al. (2022) in pawpaw fruits as they ripen and mature. Also, it is possible that slight differences in the maturity of the fruits analyzed in this study during the storage period could have accounted for the similar titratable acidity of the control fruits. From this, it is evident that the chitosan coatings effectively controlled the change in the acid content of the 10-35 fruits over time.

Table 5-3: Physicochemical properties, skin color, and textural properties of chitosan-coated North American pawpaw (*Asimina triloba*) fruits of the 10-35 cultivar during a 25-day storage at 6ºC

Treatment	Day	pH	Titratable Acidity (mg of acetic acid/100ml)	Total soluble solids (Brix)	Moisture loss (%)	L*
Control	0	6.71±0.12 ^{aA}	88.08±13.87 ^{aA}	22.38±1.00 ^{aA}	0.00±0.00 ^{dG}	48.07±2.34 ^{aAB}
	5	6.28±0.39 ^{abA}	74.73±48.92 ^{aA}	21.24±3.82 ^{aA}	2.82±0.60 ^{cCDE}	45.20±1.26 ^{aABC}
	10	6.06±0.47 ^{abA}	66.72±24.46 ^{aA}	19.51±0.70 ^{aA}	3.46±0.14 ^{cCD}	42.67±3.51 ^{abABCD}
	15	6.02±0.48 ^{abA}	61.39±30.31 ^{aA}	20.48±2.46 ^{aA}	3.82±1.08 ^{bCD}	36.80±3.90 ^{bcBCDE}
	20	5.41±0.18 ^{bA}	74.73±4.62 ^{aA}	21.18±3.30 ^{aA}	5.26±0.45 ^{abAB}	33.35±3.32 ^{cdDE}
	25	5.93±0.60 ^{abA}	66.72±16.67 ^{aA}	20.50±3.60 ^{aA}	6.62±0.74 ^{aA}	28.79±1.79 ^{dE}
1% Chitosan	0	6.61±0.33 ^{abA}	58.72±4.62 ^{aA}	18.66±1.90 ^{aA}	0.00±0.00 ^{eG}	50.96±1.52 ^{aA}
	5	6.67±0.29 ^{aA}	69.39±16.67 ^{aA}	19.80±1.88 ^{aA}	1.59±0.50 ^{dEF}	49.81±2.32 ^{aA}
	10	6.45±0.28 ^{abcA}	74.73±4.62 ^{aA}	17.97±1.87 ^{aA}	2.53±0.26 ^{cdDE}	48.43±2.53 ^{aA}
	15	6.29±0.55 ^{abcA}	66.72±25.74 ^{aA}	18.70±2.17 ^{aA}	3.12±0.31 ^{bcCD}	46.99±3.97 ^{aABC}
	20	5.72±0.19 ^{bcA}	120.10±48.70 ^{aA}	21.09±0.45 ^{aA}	4.07±0.15 ^{bBC}	45.09±4.43 ^{aABC}
	25	5.66±0.32 ^{cA}	93.41±18.49 ^{aA}	20.71±1.55 ^{aA}	5.82±0.69 ^{aA}	42.15±8.21 ^{aABCD}
2% Chitosan	0	6.56±0.49 ^{aA}	58.72±16.67 ^{aA}	19.12±2.79 ^{aA}	0.00±0.00 ^{eG}	49.64±0.45 ^{aA}
	5	6.43±0.65 ^{aA}	66.72±18.49 ^{aA}	19.49±0.96 ^{aA}	0.93±0.23 ^{dFG}	46.85±0.90 ^{abABC}
	10	6.38±0.64 ^{aA}	56.05±21.18 ^{aA}	19.70±1.93 ^{aA}	2.64±0.41 ^{cDE}	44.00±3.31 ^{abcABCD}
	15	6.03±0.66 ^{aA}	85.41±40.30 ^{aA}	20.76±1.14 ^{aA}	2.82±0.19 ^{cCDE}	40.57±3.63 ^{abcABCD}
	20	5.93±0.49 ^{aA}	80.07±27.74 ^{aA}	18.86±1.58 ^{aA}	4.14±0.36 ^{bBC}	36.56±4.92 ^{bcCDE}
	25	5.77±0.18 ^{aA}	72.06±13.87 ^{aA}	17.91±1.84 ^{aA}	5.64±0.38 ^{aA}	36.13±6.14 ^{cCDE}

Means for the parameters in each treatment that do not share a superscript are significantly different at $p \leq 0.05$.
Lowercase superscripts represent statistical differences within treatment groups for the respective treatments and uppercase superscripts represent statistical differences between all treatment groups at $p \leq 0.05$.

Table 5-3: Physicochemical properties, skin color, and textural properties of chitosan-coated North American pawpaw (*Asimina triloba*) fruits of the 10-35 cultivar during a 25-day storage at 6ºC (continued)

Treatment	Day	a*	b*	Chroma	ΔE	Hardness (kg)	Cohesiveness ratio (%)
Control	0	-7.16±0.85 ^{dDEFGH}	26.74±3.17 ^{aAB}	27.76±2.88 ^{aAB}	0.00±0.00 ^{cH}	3.7±0.5 ^{aA}	26.69±1.40 ^{bC}
	5	-4.92±1.25 ^{cdCDEFG}	25.31±2.15 ^{abAB}	25.88±1.97 ^{abABC}	6.84±3.37 ^{cEFGH}	3.8±1.4 ^{Aa}	30.60±2.06 ^{abBC}
	10	-2.51±2.62 ^{bcABCDEF}	22.78±5.02 ^{abABC}	23.08±5.03 ^{abABCD}	10.37±5.00 ^{bcCDEFGH}	3.2±1.2 ^{aA}	36.27±7.78 ^{abABC}
	15	0.97±1.87 ^{abABC}	14.36±6.13 ^{bcBCDE}	14.73±5.74 ^{bcBCDE}	19.08±7.19 ^{abABCDE}	3.3±0.9 ^{aA}	41.19±6.22 ^{aABC}
	20	3.52±1.49 ^{aAB}	8.66±4.81 ^{cdDE}	9.74±4.56 ^{cDE}	25.85±4.62 ^{aAB}	2.4±0.6 ^{aA}	32.35±2.29 ^{abABC}
	25	3.81±1.47 ^{aA}	2.59±2.86 ^{dE}	4.82±2.86 ^{cE}	29.47±0.72 ^{aA}	1.9±0.4 ^{aA}	31.78±6.86 ^{abBC}
1% Chitosan	0	-13.35±1.51 ^{bH}	28.09±1.15 ^{aA}	31.15±1.50 ^{aA}	0.00±0.00 ^{bH}	3.8±1.4 ^{aA}	32.28±7.62 ^{aABC}
	5	-13.00±1.44 ^{bH}	27.20±1.23 ^{aAB}	30.17±1.52 ^{aA}	2.94±1.16 ^{bGH}	3.3±0.5 ^{aA}	31.22±4.36 ^{aBC}
	10	-11.64±1.65 ^{abGH}	25.52±1.63 ^{aAB}	28.08±2.15 ^{aAB}	6.65±1.82 ^{abEFGH}	3.6±0.1 ^{aA}	34.94±2.19 ^{aABC}
	15	-11.06±2.36 ^{abGH}	25.58±2.94 ^{aAB}	27.91±3.62 ^{aAB}	7.96±2.29 ^{abDEFGH}	3.4±0.9 ^{aA}	36.09±9.88 ^{aABC}
	20	-9.17±2.83 ^{abEFGH}	24.74±3.65 ^{aABC}	26.46±4.38 ^{aAB}	9.10±2.69 ^{abDEFGH}	3.3±0.1 ^{aA}	37.45±1.84 ^{aABC}
	25	-4.44±5.79 ^{aBCDEFG}	18.57±8.33 ^{aABCD}	19.80±9.17 ^{aABCD}	17.25±8.58 ^{aABCDEF}	2.2±0.2 ^{aA}	44.62±0.49 ^{aAB}
2% Chitosan	0	-11.86±1.48 ^{bGH}	27.08±0.90 ^{aAB}	29.59±1.39 ^{aA}	0.00±0.00 ^{cH}	3.1±0.5 ^{aA}	32.16±2.10 ^{bABC}
	5	-10.01±1.98 ^{bFGH}	25.85±1.21 ^{aAB}	27.76±1.85 ^{abAB}	5.01±1.06 ^{bcFGH}	3.7±1.8 ^{aA}	30.48±7.15 ^{bBC}
	10	-4.42±0.59 ^{abBCDEFG}	22.02±3.79 ^{abABC}	23.11±4.59 ^{abcABCD}	11.84±1.21 ^{abcCDEFGH}	3.2±0.4 ^{aA}	40.24±5.60 ^{abABC}
	15	-4.17±3.84 ^{abABCDEFG}	18.34±4.96 ^{abABCD}	19.00±5.67 ^{abcABCD}	14.98±6.00 ^{abBCDEFG}	3.2±0.1 ^{aA}	39.49±2.28 ^{abABC}
	20	-1.43±4.02 ^{aABCDE}	14.95±5.73 ^{abABCDE}	15.40±6.08 ^{bcBCDE}	20.80±7.44 ^{aABCD}	2.3±0.4 ^{aA}	44.31±3.56 ^{aAB}
	25	0.28±3.89 ^{aABCD}	11.80±7.56 ^{bCDE}	12.37±7.48 ^{cCDE}	24.19±8.66 ^{aABC}	2.8±0.7 ^{aA}	47.38±2.78 ^{aA}

Means for the parameters in each treatment that do not share a superscript are significantly different at $p \leq 0.05$.
Lowercase superscripts represent statistical differences within treatment groups for the respective treatments and uppercase superscripts represent statistical differences between all treatment groups at $p \leq 0.05$.

In this study, fruits of different cultivars namely: Susquehanna, Sunflower, Shenandoah, Atwood, 10-35, Wells, Wilson, Prolific, NC-1, were mixed and given different treatments. The pH of the Mixed control and freshness paper-treated fruits remained statistically similar throughout the storage period until day 15 after which there were visible mold growths on the skin of the fruits and were not analyzed further (Table 5-4). The 0.001% alginate coated fruits had no significant change in pH until day 15. The pH of the fruits at days 20 and 25 were significantly different from the pH of the fruits at day 0 but not significantly different from the fruits on day 5 and day 15. Similarly, the fruits coated with the 0.005% alginate solution had no significant change in pH until day 15. The pH of the 0.005% alginate coated fruits at days 20 and 25 were significantly different from the pH of the fruits at day 0 but not significantly different from the fruits on day 5 and day 15. Despite the slight changes in pH over the storage period, the titratable acidity of all the treatments for the Mixed fruits remained statistically the same.

Table 5-4: Physicochemical properties, skin color, and textural properties of sodium alginate-coated mixed North American pawpaw (*Asimina triloba*) fruits of various cultivars during a 25-day storage at 4ºC

Treatment	Day	pH	Titratable Acidity (mg of acetic acid/100ml)	Total soluble solids (Brix)	Moisture loss (%)	L*
Control	0	6.52±0.21 ^{aABC}	58.72±16.67 ^{aA}	23.48±2.09 ^{aA}	0.00±0.00 ^{cH}	46.83±4.62 ^{aA}
	5	6.07±0.30 ^{aABCD}	82.74±12.23 ^{aA}	23.49±4.55 ^{aA}	1.35±1.05 ^{cGH}	41.19±2.59 ^{aA}
	10	6.01±0.41 ^{aABCD}	77.40±37.84 ^{aA}	25.81±3.62 ^{aA}	6.55±0.61 ^{bBCDE}	39.24±6.82 ^{aA}
	15	5.90±0.60 ^{aABCD}	82.74±33.34 ^{aA}	18.57±7.12 ^{aA}	9.99±0.90 ^{aB}	38.31±4.41 ^{aA}
Freshness paper	0	6.44±0.11 ^{aABCD}	64.06±8.01 ^{aA}	23.14±2.60 ^{aA}	0.00±0.00 ^{bH}	42.50±2.28 ^{aA}
	5	6.20±0.14 ^{aABCD}	58.72±16.67 ^{aA}	20.27±1.33 ^{aA}	1.55±0.42 ^{bFGH}	42.48±5.49 ^{aA}
	10	6.09±0.51 ^{aABCD}	66.72±36.11 ^{aA}	23.97±0.96 ^{aA}	4.73±0.97 ^{aDEFG}	42.15±6.65 ^{aA}
	15	5.76±0.14 ^{aBCD}	77.40±9.25 ^{aA}	23.13±2.70 ^{aA}	6.01±1.61 ^{aBCDEF}	36.98±5.53 ^{aA}
0.001% Alginate	0	6.53±0.06 ^{aABC}	56.05±16.01 ^{aA}	22.77±3.42 ^{abA}	0.00±0.00 ^{dH}	46.44±4.90 ^{aA}
	5	6.32±0.33 ^{abABCD}	64.06±0.20 ^{aA}	20.62±0.73 ^{bA}	2.27±0.92 ^{cdEFGH}	45.05±5.02 ^{aA}
	10	6.25±0.39 ^{abABCD}	58.72±23.11 ^{aA}	24.30±1.80 ^{abA}	5.88±1.24 ^{bcBCDEFG}	42.29±6.32 ^{aA}
	15	5.79±0.19 ^{aBCD}	93.41±32.36 ^{aA}	21.86±1.62 ^{abA}	7.68±2.74 ^{bcBCD}	38.19±6.60 ^{aA}
	20	5.68±0.33 ^{bCD}	101.42±32.36 ^{aA}	26.70±2.24 ^{aA}	10.42±2.48 ^{bB}	36.21±6.37 ^{aA}
	25	5.56±0.35 ^{bD}	104.09±8.01 ^{aA}	22.97±0.28 ^{abA}	17.34±2.89 ^{aA}	32.58±1.48 ^{aA}
0.005% Alginate	0	6.75±0.10 ^{aA}	50.71±4.62 ^{aA}	20.69±1.95 ^{aA}	0.00±0.00 ^{eH}	43.79±4.58 ^{aA}
	5	6.63±0.32 ^{abAB}	64.06±8.01 ^{aA}	22.62±1.00 ^{aA}	2.63±1.19 ^{deEFGH}	45.05±3.58 ^{aA}
	10	6.20±0.23 ^{abcABCD}	88.08±16.01 ^{aA}	23.37±1.80 ^{aA}	5.44±1.22 ^{cdCDEFG}	40.39±8.23 ^{aA}
	15	6.10±0.26 ^{bcABCD}	58.72±25.74 ^{aA}	24.58±3.00 ^{aA}	9.31±0.46 ^{bcBC}	37.63±6.63 ^{aA}
	20	6.00±0.15 ^{cABCD}	77.40±16.67 ^{aA}	23.20±0.06 ^{aA}	9.97±1.06 ^{bBC}	36.24±4.46 ^{aA}
	25	5.80±0.19 ^{cBCD}	106.76±40.30 ^{aA}	25.20±0.96 ^{aA}	15.17±2.90 ^{aA}	33.94±3.60 ^{aA}

Control and freshness paper fruits showed the presence of mold growth on the fruit skin after day 15, hence, they were not analyzed after day 15.
Means for the parameters in each treatment that do not share a superscript are significantly different at $p \leq 0.05$.
Lowercase superscripts represent statistical differences within treatment groups for the respective treatments and uppercase superscripts represent statistical differences between all treatment groups at $p \leq 0.05$.

Table 5-4: Physicochemical properties, skin color, and textural properties of sodium alginate-coated mixed North American pawpaw (*Asimina triloba*) fruits of various cultivars during a 25-day storage at 4ºC (continued)

Treatment	Day	a*	b*	Chroma	ΔE	Hardness (kg)	Cohesiveness ratio (%)
Control	0	-3.65±1.57 ^{aA}	25.07±2.57 ^{aA}	25.44±2.65 ^{aA}	0.00±0.00 ^{cC}	4.8±1.2 ^{aAB}	42.36±16.20 ^{aA}
	5	-1.15±0.77 ^{aA}	16.69±9.67 ^{aA}	17.32±9.12 ^{aA}	9.99±0.77 ^{bABC}	3.9±0.9 ^{aABC}	35.70±9.41 ^{aA}
	10	-0.44±3.87 ^{aA}	15.71±6.14 ^{aA}	16.28±5.19 ^{aA}	11.61±2.14 ^{bABC}	3.1±1.0 ^{aABC}	52.69±0.84 ^{aA}
	15	1.63±1.96 ^{aA}	15.70±5.46 ^{aA}	16.09±6.35 ^{aA}	18.85±2.80 ^{aAB}	2.8±0.2 ^{aABC}	46.05±6.78 ^{aA}
Freshness paper	0	1.39±0.86 ^{aA}	19.76±2.69 ^{aA}	20.02±2.69 ^{aA}	0.00±0.00 ^{bC}	2.9±0.1 ^{aABC}	43.47±6.65 ^{aA}
	5	-1.49±3.86 ^{aA}	14.62±8.71 ^{aA}	19.50±7.96 ^{aA}	9.88±2.90 ^{abABC}	2.7±0.1 ^{aABC}	46.90±1.99 ^{aA}
	10	-0.94±4.29 ^{aA}	18.92±7.98 ^{aA}	15.23±9.25 ^{aA}	13.96±7.83 ^{aAB}	2.8±1.3 ^{aABC}	41.73±0.52 ^{aA}
	15	1.84±1.94 ^{aA}	13.19±6.96 ^{aA}	14.02±6.87 ^{aA}	16.93±3.27 ^{a}	2.5±0.9 ^{aABC}	47.34±6.71 ^{aA}
0.001% Alginate	0	-4.50±6.01 ^{aA}	23.49±5.23 ^{aA}	24.48±6.03 ^{aA}	0.00±0.00 ^{cC}	4.1±1.3 ^{aABC}	39.47±8.10 ^{aA}
	5	-3.66±5.09 ^{aA}	23.50±4.61 ^{aA}	24.20±5.39 ^{abA}	8.27±1.79 ^{bcBC}	2.3±0.3 ^{aBC}	50.92±8.08 ^{aA}
	10	1.58±2.60 ^{aA}	13.17±9.13 ^{abA}	19.01±8.26 ^{abA}	15.92±1.76 ^{abAB}	2.6±0.2 ^{aABC}	45.72±5.42 ^{aA}
	15	-0.94±2.13 ^{aA}	18.71±8.15 ^{abA}	13.86±8.46 ^{abA}	16.32±7.90 ^{abAB}	2.0±0.4 ^{aC}	53.02±4.92 ^{aA}
	20	2.98±1.38 ^{aA}	12.99±6.13 ^{abA}	13.85±5.43 ^{abA}	18.86±4.56 ^{abAB}	2.3±0.3 ^{aC}	47.80±12.90 ^{aA}
	25	3.64±1.95 ^{aA}	5.38±1.98 ^{bA}	7.08±1.33 ^{bA}	22.39±3.87 ^{aA}	2.5±1.3 ^{aABC}	55.80±2.73 ^{aA}
0.005% Alginate	0	-1.96±2.63 ^{aA}	18.96±5.31 ^{aA}	19.32±5.55 ^{aA}	0.00±0.00 ^{bC}	3.9±0.7 ^{abABC}	36.57±2.35 ^{aA}
	5	-1.61±2.24 ^{aA}	20.48±4.15 ^{aA}	21.06±3.88 ^{aA}	8.79±3.13 ^{abBC}	5.1±0.1 ^{aA}	44.44±5.52 ^{aA}
	10	-1.74±6.44 ^{aA}	17.32±9.28 ^{aA}	18.18±9.76 ^{aA}	9.33±3.08 ^{abBC}	3.8±0.9 ^{abcABC}	36.12±18.19 ^{aA}
	15	1.01±3.12 ^{aA}	12.39±7.44 ^{aA}	13.17±6.97 ^{aA}	15.71±9.30 ^{aAB}	2.7±1.4 ^{bcABC}	53.76±2.08 ^{aA}
	20	1.60±2.10 ^{aA}	9.59±4.54 ^{aA}	10.52±4.33 ^{aA}	15.77±3.96 ^{aAB}	2.5±0.8 ^{bcBC}	58.52±8.33 ^{aA}
	25	2.91±0.62 ^{aA}	7.81±5.63 ^{aA}	8.87±5.34 ^{aA}	19.25±5.67 ^{aAB}	1.7±0.1 ^{cC}	49.15±7.47 ^{aA}

Control and freshness paper fruits showed the presence of mold growth on the fruit skin after day 15, hence, they were not analyzed after day 15.
Means for the parameters in each treatment that do not share a superscript are significantly different at $p \leq 0.05$.
Lowercase superscripts represent statistical differences within treatment groups for the respective treatments and uppercase superscripts represent statistical differences between all treatment groups at $p \leq 0.05$.

Comparing all the data obtained between the treatment groups (e.g., control day 0, 1% chitosan day 5, 2% chitosan day 10, etc.) for the respective cultivars, it is evident that the pH and titratable acidity values obtained for the Susquehanna and 10-35 fruits were not statistically different as can be seen from the ANOVA analyses shown by the uppercase superscripts in Table 5-1 and Table 5-3 respectively. However, the Sunflower fruits showed some statistical differences in the pH and titratable acidity (Table 5-2) while the Mixed fruits showed some statistical differences in only the pH values across the treatment-day combination (Table 5-4), although these most of the pH and titratable acidity values obtained were statistically similar.

5.3.2 Total soluble solids

Total soluble solids content is an important parameter in assessing fruit quality. It is a measure of the quantity of dissolved sugars and other water-soluble molecules that are present in fruit pulp. Generally, as fruits are stored over time, their total soluble solids contents increase because complex carbohydrates like pectin, cellulose, and hemicellulose from cell walls in the fruit cells breakdown into simple sugars (Islam et al., 2013; Kumar et al., 2021). Also, the change in total soluble solids content of fruits during storage could be caused by a reduction in respiration rate and an enhancement in dry matter due to moisture loss (Khorram et al., 2017).

The results from this study show that overall, there were no significant differences in the total soluble solids of the pawpaw fruits from all the cultivars and the different treatments (including control groups) during the storage period (Tables 5-1 to 5-4). This is also shown in the ANOVA analyses comparing all the treatment-day combinations for all

the cultivars shown by the uppercase superscripts. This is different from the observation Archbold et al. (2003) made that during storage there is about a 20% increase in the total soluble solids content of pawpaw fruits. From the results, it is unclear whether the treatments had any significant effect on the total soluble solids content of the fruits during storage. The total soluble solids content of the fruits in all the Susquehanna treatments in this study was similar to the value (28.0 ºBrix) reported by Brannan (2016) for the cultivar. That of the Sunflower fruits in all the treatments were also within the range of total soluble solids content in Sunflower fruits (16.0 – 20.0 ºBrix) studied by Brannan (2016) and Lolletti et al. (2021), and the values obtained for the fruits of the 10-35 cultivar throughout the storage period were higher compared to the total soluble solids content (14.64±2.32 ºBrix) reported for the cultivar by Adainoo et al. (2022).

In all the Susquehanna treatments, Sunflower chitosan treatments, 10-35 control and 10-35 2% chitosan and Mixed fruit control treatments, it was noted that the total soluble solids contents on the last day were slightly lower than the total soluble solids content on day 0. This may have been caused by the formation of alcohol during fermentation of the sugars in the pulp. However, studies have shown that the mean activity of alcohol dehydrogenase (an enzyme that catalyzes the conversion of sugars into alcohols during fruit ripening and aroma volatile formation) in pawpaw fruit does not change after harvest or during cold storage (Galli et al., 2008). Hence, it is unlikely that there was a significant alcohol production in the fruit even though the fruit it known to continue to ripen to the point of fermentation during storage after harvest. It is still crucial for future studies to analyze the fruit pulp for alcohol formation during storage to test this hypothesis. There is also the possibility that the slight reduction in total soluble solids could be due to the

complexing of simple sugars to form stringy vascular tissue that make the pulp of some of the fruits stringy during storage as was observed during the experiments on the last day for some of the fruits. This phenomenon is a physiological disorder which is accompanied by a decrease in total soluble solids that has been observed in avocadoes when they are stored for 4-6 weeks; it can be reduced by treating fruits with 1-methylcyclopropene, a plant growth regulator that inhibits ethylene action in plant cells (Choque-Quispe et al., 2022; Woolf et al., 2005). These findings provide the opportunity for further studies into the simple sugar and polysaccharide profile of the pawpaw fruit pulp during storage as this will help to optimize the storage conditions for the best pawpaw fruit quality when they are stored over a period.

5.3.3 Moisture loss

Fruits continue to respire after harvesting, taking in oxygen from the surrounding atmosphere, and releasing carbon dioxide and water in the process. This exchange of gases during fruit respiration results in loss of moisture from the fruits over time leading to a decrease in fruit weight and changes in the physical appearance and textural properties of the fruit as more moisture is lost. Studies have shown that relative humidity has a greater influence on moisture loss than storage temperature with fruits losing more moisture when they are stored at higher relative humidity (Lufu et al., 2019). Further, larger fruits are typically expected to lose less moisture over time compared to smaller fruits because the smaller fruits have a higher surface area to volume ratio. Because of this, smaller apples and eggplants lose weight faster than larger ones, and the neck area of a pear loses weight faster than the bottom part of the fruit (Lufu et al., 2020). Many other factors also affect the rate of moisture loss in fruits during postharvest storage including cultivar, orchard

practices, fruit peel thickness, weather conditions, harvesting techniques and mechanical injuries, airflow during storage, and packaging (Lufu et al., 2020).

The data obtained show that for the Susquehanna fruits, the chitosan coatings successfully controlled the loss of moisture in the fruits compared to the control fruits as shown by the ANOVA analyses within the treatment groups and between the treatment groups. By day 5, the control fruits had already lost 7.20±0.52% moisture meanwhile the 1% chitosan (6.94±0.86%) and 2% chitosan (6.86±0.79%) fruits lost similar amounts by day 25 (Table 5-1). Similarly, the chitosan coatings were effective in controlling moisture loss in Sunflower fruits as can be seen from the ANOVA analyses within the treatment groups and between the treatment groups in Table 5-2. The control Sunflower fruits had lost 7.39±1.35% moisture by day 15, but by day 25, the 1% chitosan coated fruits had lost only 3.97±0.23% and the 2% chitosan coated fruits had lost 3.70±0.62% (Table 5-2). This further indicates that for the Sunflower fruits, the 2% chitosan coating better controlled moisture loss than the 1% chitosan coating. For the 10-35 fruits, although the control fruits had averagely lost more moisture by day 25 the variation in moisture loss of the control fruits makes the moisture loss in the control fruits similar to the moisture loss in the chitosan coated fruits, meaning the chitosan coatings did not significantly affect moisture loss in 10-35 fruits compared to the control fruits as can be seen from the ANOVA analyses within the treatment groups and between the treatment groups (Table 5-3).

The fruits in the mixed fruit group lost more moisture during the storage period compared to the moisture lost by the fruits in the respective cultivar groups. Nonetheless, the data show that mixed fruits with the freshness paper treatment (6.01±1.61%) lost less moisture within the 15-day period of storage than the control fruits (9.99±0.90%) before

both groups had visible mold growths on the skin of the fruits (Table 5-4). From the one-way ANOVA within the treatment groups (the lowercase superscripts by the values in the tables), it is evident that for the freshness paper treatment, there were relatively fewer changes in the moisture loss over time compared to the sodium alginate coated fruits. This suggests that the sodium alginate coatings were not very effective in controlling the loss of moisture from the fruits during storage. This may have been due to the hydrophilicity of alginate and the fact that the water vapor permeability of alginate films (390 $g \cdot m^{-1} \cdot s^{-1} Pa^{-1}$) is higher than that of chitosan films (360 $g \cdot m^{-1} \cdot s^{-1} Pa^{-1}$) (ALSamman & Sánchez, 2022; Vargas et al., 2008). Further, the low concentration of alginate used in the experiments than what has been used in other literature could account for the higher moisture loss. However, in the pre-experiment trials, 1% alginate and 2% alginate coatings were tested but these coatings did not adhere to the fruit well after drying, which is why lower concentrations (0.001% and 0.005%) which adhered to the fruits were used in this study.

The fruits in the mixed fruit group lost more moisture during the storage period compared to the moisture lost by the fruits in the respective cultivar groups. Nonetheless, the data show that mixed fruits with the freshness paper treatment (6.01±1.61%) lost less moisture within the 15-day period of storage than the control fruits (9.99±0.90%) before both groups had visible mold growths on the skin of the fruits (Table 5-4). From the one-way ANOVA and Tukey's test (the superscripts by the values in the tables), it is evident that freshness paper treatment there was relatively fewer changes in the moisture loss over time compared to the sodium alginate coated fruits. This suggests that the sodium alginate coatings were not very effective in controlling the loss of moisture from the fruits during storage. Studies have shown that although polysaccharide-based (e.g., alginate) edible

coatings offer good barrier properties, they are hydrophilic with a high-water vapor permeability (Vargas et al., 2008). This may have accounted for the high moisture loss in the alginate coated fruits. Also, the hydrophilicity of alginate and the fact that the water vapor permeability of alginate films (390 $g \cdot m^{-1} \cdot s^{-1} Pa^{-1}$) is higher than that of chitosan films (360 $g \cdot m^{-1} \cdot s^{-1} \cdot Pa^{-1}$) could explain why the alginate coated fruits lost more moisture over time than the chitosan coated fruits (ALSamman & Sánchez, 2022; Vargas et al., 2008). Further, the low concentration of alginate used in the experiments compared with levels used in other literature could account for the higher moisture loss. However, in the pre-experiment trials, 0.5% alginate, 1% alginate and 2% alginate coatings were tested but these coatings did not adhere to the fruit well after drying, which is why lower concentrations (0.001% and 0.005%) which adhered to the fruits were used in this study.

Moisture loss in fruits occurs by the loss of moisture from the peel which is continuously replenished by migration of moisture from the pulp (Singh and Reddy, 2006). Based on this, it is expected that fruits with thicker peels lose less moisture compared to those with thinner peels since moisture can migrate across thinner peels easily and more rapidly. Also, with the added edible coating, it is expected that the edible coating will increase the barrier moisture has to travel across to leave the fruit, thereby slowing moisture loss. Previous studies have found that Susquehanna fruit have a peel thickness of 0.51±0.18 mm, Sunflower fruits have a peel thickness of 0.34±14 mm and 10-35 fruits have a peel thickness of 0.38±0.18 mm (Adainoo et al., 2022). However, these peel thickness values do not correspond to the percent moisture loss for these cultivars. Other factors such as airflow during storage may have had a more significant effect on the percent moisture loss from the Susquehanna, Sunflower and 10-35 fruits than their respective peel thickness.

5.3.4 Skin color and physical appearance

One of the main challenges that has made commercializing the North American pawpaw difficult has been the rapid color change that occurs in the skin of the fruit during postharvest storage. It is therefore critical that technologies aimed at extending the shelf life of the fruits also control the change of the skin color over time, since the skin color is an important indicator of pawpaw fruit quality. One of the key color parameters for monitoring the changes in skin color is the ΔE value. According to Bhookya et al. (2020), ΔE values greater or equal to 2 can be detected by the human eye, but ΔE values less than 0.3 cannot be detected by the human eye.

Data obtained from the experiments show that for the Susquehanna control fruits, there were significant differences in the color parameters (L*, a*, b*, chroma and ΔE) ($p<0.05$), however, the 1% chitosan and 2% chitosan coatings were effective in controlling the change of the skin color with no statistically significant variation in L*, a*, b*, chroma and ΔE values during the 25-day storage period (Table 5-1). Although from Figure 1, it can be seen that the Susquehanna 1% and 2% chitosan fruits were darker in skin color on day 25 compared to their skin color on day 0. Further, there was a similar observation in the Sunflower fruits. The Sunflower control fruits showed statistically significant ($p<0.0001$) changes in skin color parameters, but the chitosan coated fruits showed no statistically significant ($p>0.05$) changes in skin color parameters over the storage duration (Table 5-2) except for the ΔE values of the Sunflower 1% chitosan fruits that had some statistically significant ($p=0.018$) changes during the storage. Like the other cultivars, the 10-35 control fruits had statistically significant ($p<0.0001$) changes in color parameters during the storage. The 10-35 1% chitosan fruits had no statistically significant ($p>0.05$)

changes in color parameters over time except for the a* values and ΔE values which changed significantly ($p<0.05$) (Table 5-3). The 10-35 2% chitosan fruits had statistically significant changes in color parameters throughout the storage ($p<0.05$). The Mixed control and freshness paper fruits had no statistically significant changes in all the color parameters except the ΔE values, which changed significantly ($p<0.01$) during storage (Table 5-4). The 0.001% alginate was effective in controlling changes in only the b*, chroma and ΔE values but the 0.005% alginate coating effectively controlled the changes in all the color parameters (Table 5-4).

Both 1% chitosan and 2% chitosan coatings were able to delay the molding of the fruits, which occurred after day 15 for the control Susquehanna, control Sunflower, control mixed and freshness paper treated fruits. This may be attributed to chitosan's natural antifungal and antimicrobial properties, which is also dependent on factors such as molecular weight, the influence of the fruit on which it is applied and the components of the chitosan coating solution (Devlieghere et al., 2004; Zheng & Zhu, 2003). In lettuce, the antimicrobial effect of chitosan disappears after 4 days of storage, meanwhile, in strawberries, it takes 12 days for the antimicrobial effect of chitosan to disappear (Devlieghere et al., 2004). In this study, while microbiological tests were not performed on the coatings, there were physical observations of mold growth on the skin of the chitosan-coated fruits after day 25 as can be seen in Figure 1. From this, it could be said that the antimicrobial effect of chitosan disappeared after day 25. Future studies could further explore how interactions with the pawpaw fruit affect chitosan's antimicrobial activity. Despite these findings, the 10-35 control fruits had no physical observations of mold

growth throughout the storage period, which may be due to a genetic trait of the cultivar that enables it to resist mold growth for a period.

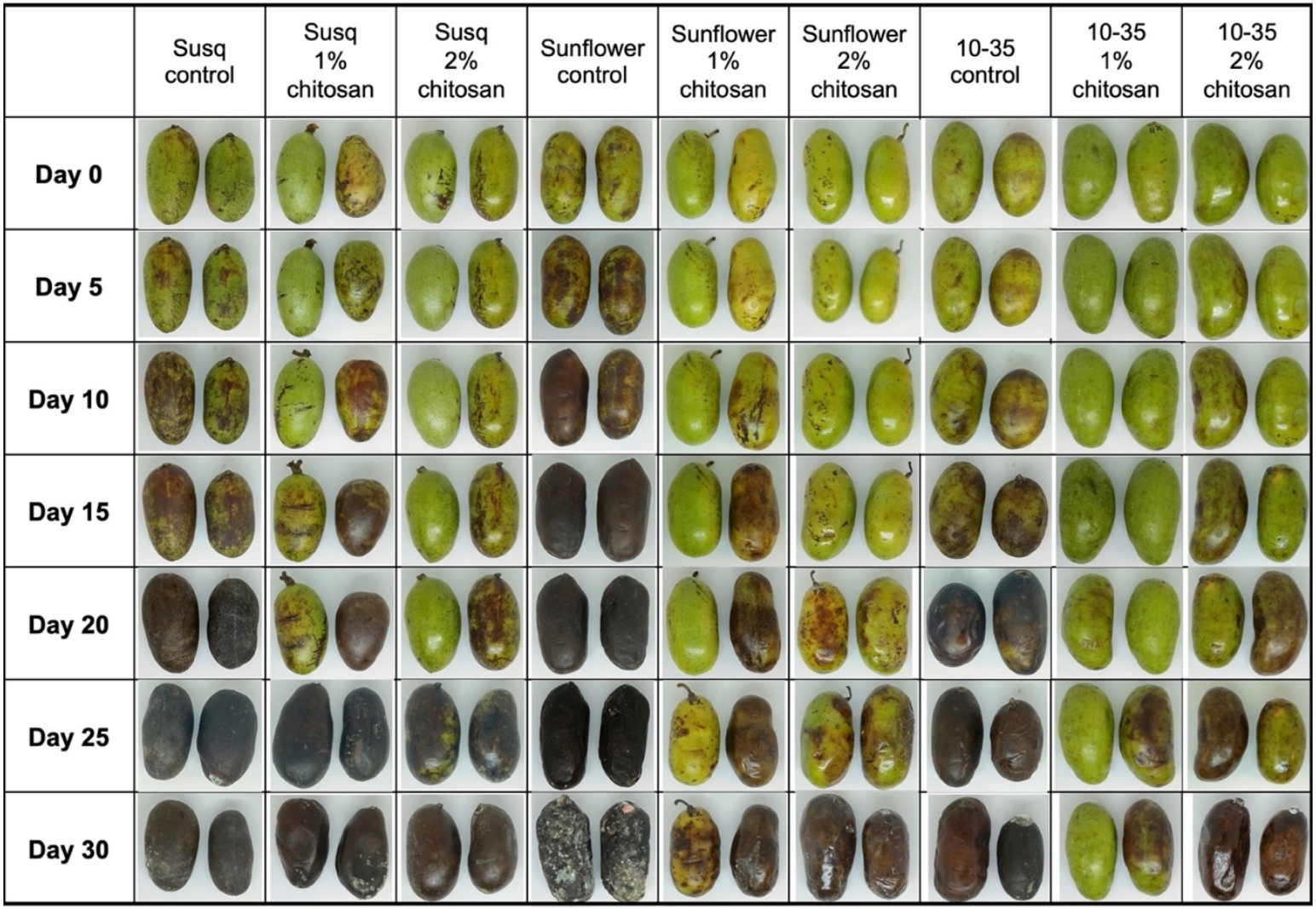

Figure 5-1: Images showing the physical appearance of pawpaw fruit samples for the Susquehanna, Sunflower and 10-35 cultivars and their chitosan treatments during storage. (Susq = Susquehanna)

5.3.5 Textural properties

The North American pawpaw fruit is known to be a climacteric fruit which continues to ripen postharvest to the point where it is too soft to handle, suggesting that its cohesiveness ratio (the strength of the internal bonds of the fruit) reduces as it ripens. This is one of the reasons why it has remained challenging for the fruit to be marketed since these changes in textural properties all happen within 5 days after harvest (Archbold et al., 2003; Galli et al., 2008). In addition, pawpaw ripeness is typically determined by the hardness (or firmness) of the fruit. These make the textural properties; hardness and cohesiveness ratio, crucial for determining fruit quality. According to Archbold et al. (2003), pawpaw fruits can be stored at 4ºC for 1 month with little change in firmness, however, in cherimoya fruits (another fruit in the Annonaceae family), it was found that storage at temperatures below 7ºC resulted in chilling injury, which affects textural properties.

Among the Susquehanna fruit treatments, the 1% chitosan coating better controlled the change in hardness of the fruits than the 2% chitosan coating, although in both chitosan treatments there was a reduction in hardness with time (Table 5-1). For all the Susquehanna fruits, it was found that the cohesiveness ratio increased with time (Table 5-1). For the Sunflower fruits, there were statistically significant changes in the hardness and cohesiveness ratio of fruits in both 1% and 2% chitosan coating treatments (Table 5-2). The 10-35 fruits, like the Susquehanna fruits, had no statistically significant change in hardness throughout the storage period, even though there was a decrease in hardness (Table 5-3). Among the 10-35 fruit treatments, 1% chitosan coating was more effective in controlling the change in cohesiveness ratio during storage than the 2% chitosan coating.

The mixed fruits showed no statistically significant change in both hardness and cohesiveness ratio throughout the storage period, except for the 0.005% alginate coated fruits which showed some variations in hardness during storage (Table 5-4).

Overall, the hardness of the fruits reduced, and the cohesiveness ratio increased with time. The increase in may have resulted from the loss of moisture which increased the strength of the internal bonds within the fruits.

5.3.6 Principal component analysis of variability

Principal component analysis (PCA) is a multivariate analysis technique that helps to reduce the dimensionality of the data matrix, provide insights into the relationship between the quality characteristics studied, highlight the differences between the quality characteristics of pawpaw fruits, and enable the visualization of the multidimensional data. In this study, PCA was also performed to identify clusters among the different treatments studied based on their similarities. According to Boateng et al. (2021), a total variance of 70-90% is desirable to explain the variability in a principal component analysis. The results show that the rate of variance contribution of the first, second, and third PCs were 50.5%, 15.1%, and 11.5% respectively. The total rate of variance contribution of the first three PCs was 77.1%. Hence, the first three principal components (PC1, PC2, and PC3) are enough to explain most of the total variance of the pH, titratable acidity, total soluble solids, moisture loss, color parameters (L*, a*, b*, chroma and ΔE), and textural properties (hardness and cohesiveness ratio) can be explained by the first three PCs.

Based on the extracted eigenvectors, PC1 was contributed to by a* values (-0.356), ΔE (-0.331), moisture loss (-0.323), L* values (0.388), b* values (0.393), and chroma

(0.394); PC2 was contributed to by pH (-0.417), total soluble solids (0.511), and titratable acidity (0.605); and PC3 was contributed to by hardness (-0.579), and cohesiveness (0.623). From the PCA loading plots (Figure 5-2A-C), it is evident that based on the data obtained in this study, there are some correlations between some of the quality parameters analyzed. Figure 2A shows that there are significant correlations between moisture and cohesiveness ratio, and titratable acidity and total soluble solids, although these correlations are weak as can be seen from the Pearson's correlation coefficients in Table 5. Further, there are significant correlations between b* value and chroma, and a* and ΔE values as can be seen in Figure 5-2A and 5-2B. The biplot (Figure 5-2D) shows that comparing all the controls and the treatments, there were similarities in the effect of the treatments studied. This is shown in the overlap of the 95% confidence ellipses for the treatments. Pooling all the data together, beyond the correlations, PCA shows that there is no clear separation in the effect of the chitosan, alginate, and freshness paper treatments from the control groups based on the quality characteristics studied.

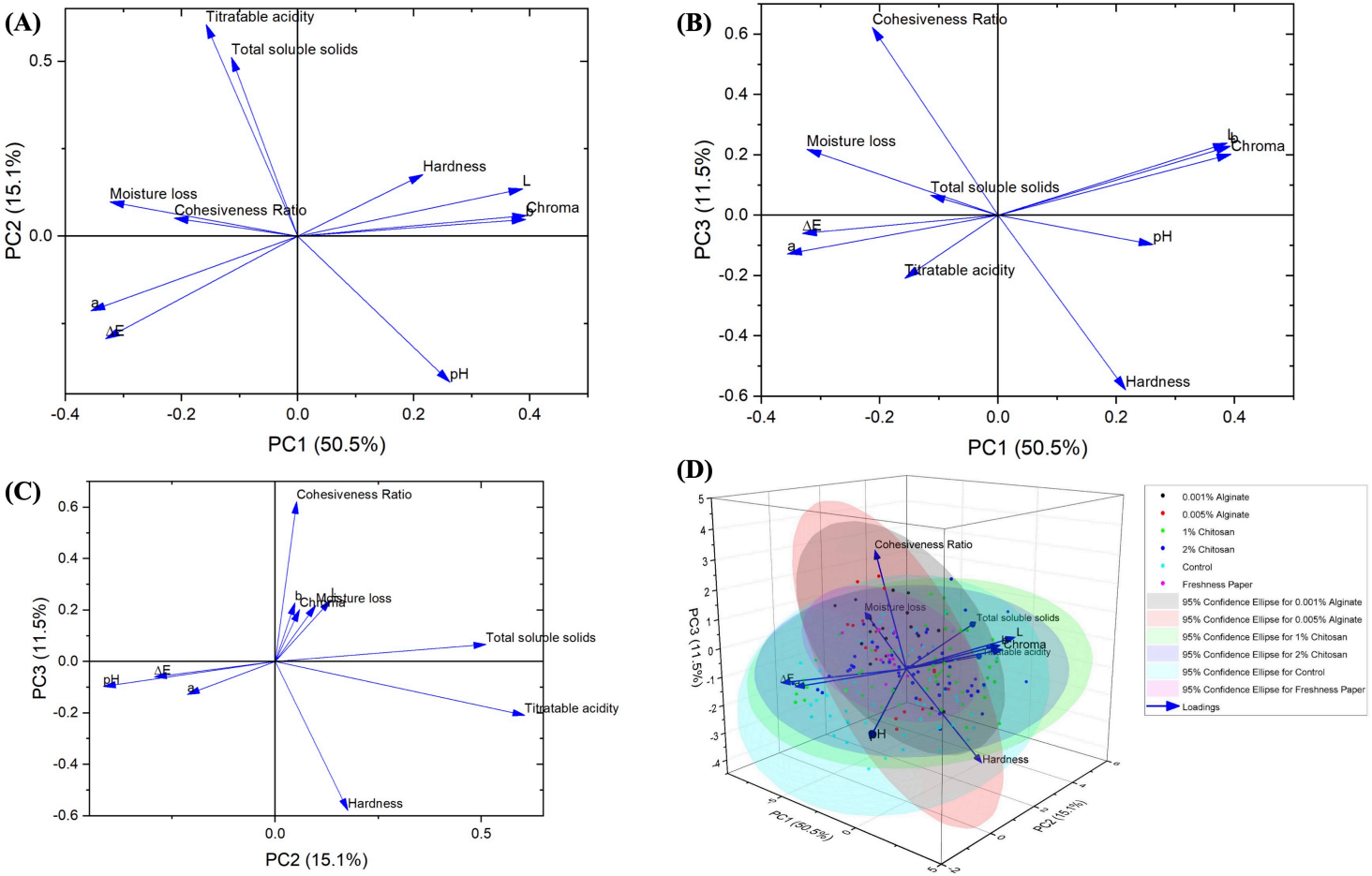

Figure 5-2: 2D loading plots (A-C) and 3D biplot (D) from the principal component analysis of the quality characteristics of the pawpaw fruits during storage

Table 5-5: Correlation matrix of Pearson's correlation coefficients (r) for the quality characteristics of the pawpaw fruits

	pH	Titratable acidity	Total soluble solids	Moisture loss	L*	a*	b*	Chroma	ΔE	Hardness	Cohesiveness Ratio
pH	1.00	-0.64	-0.29	-0.50	0.41	-0.30	0.48	0.46	-0.36	0.31	-0.34
Titratable acidity	-0.64	1.00	0.38	0.32	-0.26	0.07	-0.33	-0.32	0.06	0.04	0.04
Total soluble solids	-0.29	0.38	1.00	0.25	-0.13	0.17	-0.21	-0.21	-0.07	0.01	0.25
Moisture loss	-0.50	0.32	0.25	1.00	-0.59	0.51	-0.60	-0.62	0.53	-0.45	0.48
L	0.41	-0.26	-0.13	-0.59	1.00	-0.85	0.94	0.93	-0.73	0.33	-0.28
a	-0.30	0.07	0.17	0.51	-0.85	1.00	-0.80	-0.80	0.72	-0.32	0.37
b	0.48	-0.33	-0.21	-0.60	0.94	-0.80	1.00	0.95	-0.70	0.32	-0.31
Chroma	0.46	-0.32	-0.21	-0.62	0.93	-0.80	0.95	1.00	-0.70	0.34	-0.33
ΔE	-0.36	0.06	-0.07	0.53	-0.73	0.72	-0.70	-0.70	1.00	-0.46	0.24
Hardness	0.31	0.04	0.01	-0.45	0.33	-0.32	0.32	0.34	-0.46	1.00	-0.50
Cohesiveness Ratio	-0.34	0.04	0.25	0.48	-0.28	0.37	-0.31	-0.33	0.24	-0.50	1.00

5.3.7 Ranking the treatments using the TOPSIS-Shannon entropy method

In order to further test which treatment performed best for the respective cultivar groups, TOPSIS-Shannon entropy method was employed. TOPSIS (technique for ordered preference by similarity to ideal solution) is a multicriteria decision making analysis that ranks the alternatives based on their distance from the ideal solution (Khodaei et al., 2021).

In this study, the mean of each of the quality parameters analyzed were selected as important criteria in assessing the quality of the fruits during the storage period. Total soluble solids, pH, L* values, b* values, chroma, hardness, and cohesiveness ratio were considered as the positive criteria since higher values are preferred for these quality characteristics while titratable acidity, moisture loss, a* values, and ΔE values were considered as the negative criteria since lower values are preferred for these quality characteristics. The data were normalized and the weight for each of these criteria was determined by the Shannon entropy method (Table 5-6). The a* values had the highest influence (0.6533) on the quality of the pawpaw fruits during storage while pH had the least influence (0.0009) on the quality of the pawpaw fruits during storage.

Table 5-6: Criteria and suggested weights for each response by Shannon entropy method

Criteria	Weight
a*	0.6533
Moisture loss	0.1073
Titratable Acidity	0.0615
b*	0.0528
Chroma	0.0499
ΔE	0.0386
Cohesiveness ratio	0.0165
L*	0.0078
Hardness	0.0069
Total soluble solids	0.0046
pH	0.0009

Table 5-7: Final rankings of the treatments on improving the shelf-life of North American pawpaw fruits obtained by TOPSIS analyses

Cultivar	Treatment	S_i^+	S_i^-	$S_i^+ + S_i^-$	P_i	Rank
10-35	1% Chitosan	0.010	0.437	0.447	0.978	1
Susquehanna	2% Chitosan	0.172	0.267	0.439	0.609	2
Sunflower	2% Chitosan	0.172	0.267	0.439	0.609	2
10-35	2% Chitosan	0.212	0.226	0.438	0.517	4
Sunflower	1% Chitosan	0.255	0.186	0.440	0.422	5
Susquehanna	Control	0.275	0.163	0.438	0.372	6
Susquehanna	1% Chitosan	0.325	0.113	0.438	0.258	7
10-35	Control	0.385	0.058	0.443	0.132	8
Mixed	Control	0.391	0.051	0.442	0.116	9
Mixed	Freshness Paper	0.435	0.033	0.469	0.071	10
Sunflower	Control	0.422	0.024	0.446	0.054	11
Mixed	0.001% Alginate	0.423	0.020	0.442	0.044	12
Mixed	0.005% Alginate	0.430	0.016	0.446	0.036	13

The performance scores of the treatments for the respective cultivar groups were determined using their distances from the ideal (S_i^+) and negative points (S_i^-) as shown in Table 5-7. From this, the treatments were ranked based on their performance scores, and it was found that for 10-35 fruits, 1% chitosan coating performed better than the 2% chitosan coating, which performed better for Susquehanna and Sunflower fruits. The effect of the 1% chitosan coating on the 10-35 fruits can even be seen in the physical appearance of the fruits as shown in Figure 5-1. Apparently, the 10-35 fruits coated with 1% chitosan had a more controlled physical appearance than the fruits in the other treatment groups. This was followed by the Sunflower 2% chitosan and Susquehanna 2% chitosan fruits. These findings further indicate that chitosan coatings are effective for controlling changes in the quality of pawpaw fruits possibly because of how chitosan strongly alters carbon metabolism in the fruits thereby positively influencing the quality characteristics during storage (Cosme Silva et al., 2017). As expected, the control fruits performed worse than the chitosan coated fruits except for the Susquehanna 1% chitosan coated fruits. Also, comparing the freshness paper treated fruits to the other treatments, it is evident that overall the freshness paper treatment did not perform well in maintaining the quality and its performance was worse than the Mixed control fruits but slightly better than the Sunflower control fruits. In addition, the alginate coated fruits performed the worst compared to all the treatments, which may be due to the low concentration of alginate used in the coating solutions. Also, the complex effect of the differences in the metabolic processes of the fruits from different cultivars used for the alginate coating experiments could have had an impact on the effectiveness of the alginate coatings. In the future, improved alginate

coating formulations could be tested on fruits from specific pawpaw cultivars to confirm their effect on the quality characteristics of pawpaw fruits during storage.

5.4 Conclusion

The findings from this study show that edible coatings have effects on the quality characteristics of pawpaw fruits during storage. However, the effect of the edible coatings varied for different quality characteristics during the storage period. Freshness paper treatment also had some effect on the quality of pawpaw fruits during storage. The chitosan coatings were more effective in slowing moisture loss in Sunflower than in Susquehanna and 10-35 fruits, and the freshness paper treatment better controlled moisture loss than the alginate coatings. Although the treatments generally controlled the change in pH, acidity, and total soluble solids, there were variations in some color parameters as well as textural properties over time. Further, the chitosan and alginate coatings delayed mold growth on the skin of the pawpaw fruits during the storage period. The TOPSIS analyses revealed that 1% chitosan coatings are effective in maintaining the quality of 10-35 pawpaw fruits while 2% chitosan is better for Sunflower and Susquehanna pawpaw fruits. To our knowledge, this is the first scientific study conducted to investigate the effects of edible coating on extending the shelf-life of pawpaw fruit. The experimental data from different cultivars, treatments, and storage conditions, proved the shelf-life of pawpaw fruit could be extended from 5 days to 15-20 days depending on the cultivar. These findings have a greater significance from a food processing standpoint and can help in the selection and use of whole pawpaw or pawpaw as a food ingredient for different food applications. Also this

will pave the way for whole pawpaw fruits with longer shelf lives to be commercialized, creating new markets for this underutilized specialty crop.

Overall Summary and Recommendations

This study was aimed at studying the physical properties of the North American pawpaw fruits to assess its processing potential, and identify noninvasive ripeness indicators. In this study, experiments on the preservation of the whole pawpaw fruit were conducted. The findings from this study provide new insights into some of physical properties of the pawpaw fruits which had previously not been studied such as peel thickness which is an important parameter for understanding how to handle the fruit postharvest to prevent postharvest losses. This study also identified some cultivars that are better suitable for specific processing operations, like the Susquehanna cultivar which may potentially be more suitable for pulp processing, while Overleese may potentially be more suitable for juice production. Further, the new findings from this study challenged the popular notion that skin color is a poor indicator of pawpaw fruit ripeness. The results obtained from the study show that there are strong correlations between the skin color greenness and the textural properties, and between the skin color hue angle and the textural properties. With these findings, pawpaw farms can potentially be monitored with unmanned aerial vehicles to ensure prompt harvesting of the fruits, without the use of invasive textural properties and harvesting practices which might bruise the fruits and result in rapid decline in fruit quality contributing to postharvest losses. In addition, the experiments on extending the shelf life of the whole pawpaw fruit shows that chitosan edible coatings can be used to extend the shelf life of the fruit from 5 days to 15-20 days depending on the cultivar.

Despite the progress made in this study, it is crucial for researchers to conduct further studies on various quality characteristics of the pawpaw fruit to gain a deeper

understanding of the changes that occur in the fruit during maturation, ripening, postharvest storage and processing. Since one key characteristic of the pawpaw fruit is its aroma when it is ripe, it is very important for future studies to investigate the changes in flavor and aroma volatiles during postharvest storage as this could also be used as a noninvasive measure of pawpaw ripeness. Also, the paucity of data on the rate of respiration and ethylene production of pawpaw fruits of different cultivars should encourage further studies of these properties since data on these could be used to develop modified atmosphere systems to extend the shelf life of the fruit.

Finally, to design improved storage conditions for pawpaw fruits, it is critical to understand the thermophysical properties of the fruit. Currently, there is no published data on the thermophysical properties such as thermal conductivity of the pawpaw fruit. These parameters are needed for the determination of the thermal diffusivity, which is an indicator of how fast a material stores or releases heat. The thermophysical properties of most foods are often obtained using mathematical prediction models. However, mathematical prediction models are generic and not very reliable, hence, experimentally determining the thermophysical properties of pawpaw will provide a solid basis for the design of storage techniques to ensure prolonged shelf life.

Appendix

The tables below show the extracted eigenvectors for the 3D principal component biplots that were generated from the principal component analyses of the data obtained in Chapter 2 (Table A-1) and Chapter 3 (Table A-2).

Table A-1: Extracted eigenvectors for the 3D principal component biplot of the ripe and unripe North American pawpaw fruits

	Coefficients of PC1	Coefficients of PC2	Coefficients of PC3
L fruit	-0.17194	0.38204	0.17933
a fruit	0.26595	-0.11442	0.18414
b fruit	-0.03048	0.58642	0.15678
Chroma fruit	-0.16489	0.46949	0.11869
Delta E fruit	0.27652	-0.01981	0.10081
Hue Angle fruit	0.20935	-0.2971	0.06351
L pulp	-0.25466	-0.0643	0.10519
a pulp	0.27415	0.05523	0.08406
b pulp	0.27035	0.08382	0.09804
Chroma pulp	0.27016	0.08459	0.09111
Delta E pulp	0.27683	0.06968	0.06803
Hue Angle pulp	0.27627	0.08801	0.0829
Hardness	-0.25191	0.00307	-0.30814
Resilience ratio	-0.14651	-0.16111	0.59196
Cohesiveness ratio	-0.24799	-0.15144	0.28567
Springiness ratio	-0.09962	-0.29467	0.2776
Chewiness	-0.26251	-0.08914	-0.03347
Adhesiveness	-0.10753	0.01955	0.46901
Total soluble solids	0.27466	0.09978	0.09166
Total soluble solids	0.2658	0.09861	0.08354

Table A-2: Extracted eigenvectors for the 3D principal component biplot of the North American pawpaw fruits with edible coatings and freshness paper treatments

	Coefficients of PC1	Coefficients of PC2	Coefficients of PC3
pH	0.26225	-0.41669	-0.09716
Titratable acidity	-0.15793	0.60517	-0.20899
Total soluble solids	-0.11403	0.51104	0.06639
Moisture loss	-0.32343	0.09825	0.21863
L	0.38771	0.13544	0.23957
a	-0.35631	-0.21319	-0.12806
b	0.39294	0.04821	0.22907
Chroma	0.39434	0.0593	0.20249
ΔE	-0.33106	-0.29305	-0.06049
Hardness	0.21605	0.17642	-0.57948
Cohesiveness Ratio	-0.21264	0.05184	0.62286

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