Journal of Food Research; Vol. 6, No. 3; 2017
ISSN 1927-0887 E-ISSN 1927-0895
Published by Canadian Center of Science and Education

Effect of Frozen Storage on Polyphenol Oxidase, Antioxidant Content, and Color of Pawpaw (*Asimina Triloba* [L.] Dunal) Fruit Pulp

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Received: January 6, 2017 Accepted: January 28, 2017 Online Published: May 10, 2017

doi:10.5539/jfr.v6n3p93 URL: https://doi.org/10.5539/jfr.v6n3p93

Abstract

Polyphenol oxidase (PPO) activity values for pawpaw pulp during frozen storage were measured for the main effect of month of storage at three levels (0, 4, 8 months) and treatment at four levels (vacuum, air, ascorbic acid or n-acetylcysteine). A significant effect of treatment was observed in PPO activity ($p<0.001$). *Post hoc* analysis revealed no significant difference between samples that were vacuum packaged and those for which no attempt to exclude air was made. The addition of the two chemical browning inhibitors significantly lowered PPO activity. Ascorbic acid exhibited a significant 69% reduction in PPO activity compared to vacuum and air samples and n-acetylcysteine was significantly more effective than ascorbic acid and almost completely inhibited PPO activity compared to the vacuum and air samples. CIELAB tristimulous color values (L*, a*, b*) were used to generate the applied color values total color difference (ΔE), browning index, hue and chroma in pawpaw pulp for the two main effects. Analysis of variance for the main effects showed significance for all seven color attributes at $p<0.001$. For the main effect of storage time, ANOVA showed significance during storage for all seven color attributes at $p<0.001$, indicating that there were color changes during storage. Pawpaw pulp samples at 8 months of storage were significantly darker (lower L*), more yellow (higher b*), more vivid (higher chroma), and had a higher browning index than the samples at 0 or 4 months of storage. For the main effect of treatment, ascorbic acid and n-acetylcysteine treatment produced pawpaw pulp that was significantly different than samples to which air was not excluded for all seven dependent color variables. Specifically, n-acetylcysteine and ascorbic acid produced pulp that was lighter (higher L*), less red (lower a*), and more yellow (higher b* and hue), more vivid (higher chroma), and exhibited more color difference (higher ΔE). A strategy to inhibit enzymatic browning during frozen storage would be useful for the nascent pawpaw industry.

Keywords: pawpaw; polyphenol oxidase, frozen storage, n-acetylcysteine, ascorbic acid

1. Introduction

This article is the third in a series in this journal about pawpaw (*Asimina triloba* L. Dunal) fruit, one of only a few members of the tropical Annonaceae family that is a temperate species. The first dealt with pawpaw fruit flavor and color and reported that pawpaw flavor resembles a combination of banana and mango (Brannan, Salabak, & Holben, 2012). The second characterized polyphenol oxidase (PPO) from different pawpaw varieties (Brannan, 2016).

PPO is responsible for enzymatic browning in fruits and currently there are two published studies on the activity of PPO in pawpaw. Researchers have identified two isoforms of pawpaw PPO (EC 1.10.31) of 28.2 and 38.3 kDa and the pH (6.5-7.0) and temperature (5-20^{o}C) for optimum activity (Fang, Wang, Xiong, & Pomper, 2007). The activity and kinetic parameters of PPO in 12 varieties of pawpaw pulp (Brannan, 2016) have been characterized. Substrates for PPO include many polyphenolic compounds that have been identified in pawpaw pulp (Brannan, Peters, & Talcott, 2015).

Pawpaw fruit pulp is susceptible to rapid post-harvest increases in tissue browning and fruit softening (McGrath & Karahadian, 1994). The 3-7 day shelf life of pawpaw fruit that is ripened on the tree (Layne, 1996) can be extended for up to several weeks by refrigeration (Archbold, Koslanund, & Pomper, 2003). Refrigeration of ripe pawpaw pulp exhibits no effect on phenolics, flavonoids, reducing potential, and radical scavenging compared to fresh pulp (Harris & Brannan, 2009), however, differences were observed in these attributes during 300 d of

frozen storage.

Ascorbic acid is found in many fruits and can function as an important enzymatic browning inhibitor. Ascorbic acid deactivates PPO activity by chelating copper from the prosthetic group of PPO. Ascorbic acid also inhibits enzymatic browning by reducing quinones back to phenols (Limbo & Piergiovanni, 2006). Although pawpaw pulp has been reported to contain 4.98 mg ascorbic acid per 100 g of fresh pulp, (Harris & Brannan, 2009)), enzymatic browning is not completely inhibited (Fang, Wang, Xiong, & Pomper, 2007).

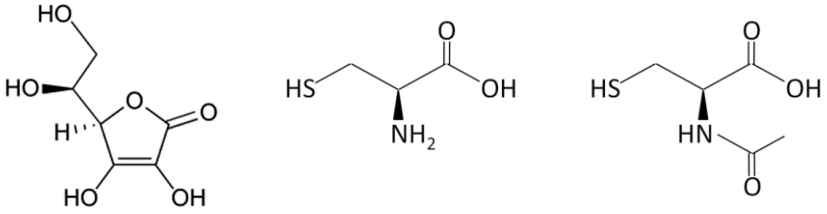
N-acetylcysteine (NAC), a derivative of L-cysteine, is naturally-occurring in fruits such as lemon, grape, and strawberry. NAC content has not been measured in pawpaw. NAC is a potential PPO inhibitor because it can react with quinones at the initial stage of the enzymatic browning reaction to reduce O-quinones to O-diphenols, producing colorless products (Demirkol, Adams, & Ercal, 2004). NAC has been used as a browning inhibitor in pears, with 0.75% of NAC in combination with glutathione preventing enzymatic browning on fresh-cut pears for up to 28 days at 4℃ (Oms-Oliu, Aguilo-Aguayo, & Martin-Belloso, 2006).

Frozen pawpaw pulp is commercially available and usually includes ascorbic acid as a browning inhibitor. Nonetheless, frozen tissue browns very easily upon thawing and longer term frozen storage. The objective of this research was to characterize PPO activity, color, and antioxidant content of pawpaw pulp during 8 months of frozen storage.

2. Method

2.1 Sample Preparation

Pawpaws were collected from a single tree in Athens, Ohio. Although these pawpaws are considered "wild," they have unofficially come be known as variety *Rana* because the tree has produced pawpaws that earned top honors in the "Best Pawpaw Contest" at the yearly Ohio Pawpaw Festival based on their weight, appearance, skin surface, aroma, skin thickness, flavor, texture, aftertaste, and number of seeds. The pawpaw pulp was separated from the skins and seeds and then the pulp was pooled. The pulp was separated into four portions, two of which were unadulterated and two of which had ascorbic acid or NAC (a cysteine analog shown in Figure 1) added to achieve a final concentration of 1%. Once portioned, the pawpaw pulp was placed into polyethylene/nylon FoodSaver (Jarden Corp., Rye, NY) 27.94-cm bags with an oxygen transmission rate of 6.7 cc/m^2h/23 ℃/0% RH. The bags were randomly selected prior to labeling. Once the bags were filled, they were either vacuum sealed (vacuum storage) or sealed without attempting to remove air prior to sealing (air storage, ascorbic acid, NAC), then immediately transferred into frozen storage at -18 ℃. Samples at 0 month and at subsequent 4-month intervals, pawpaw samples were immediately transferred from -18 ℃ to a freezer at -40 ℃ to be analyzed at a later time.



Ascorbic Acid Cysteine N-Acetyl Cysteine

Figure 1. Structure of ascorbic acid, cysteine, and N-acetyl cysteine.

2.2 Crude Pawpaw Pulp Extraction

Crude extracts from the treated pawpaw fruit pulp were prepared in a 1:5 (w/v) ratio (pulp/methanol) using a Waring blender followed by gentle agitation for 1 hour. No further extraction was performed. The extracts were filtered and stored at -40^{o}C.

2.3 Measurement of Color, Polyphenol Oxidase, Total Phenolics, Total Flavonoids

Pulp color was characterized by using a Konica Minolta Colorimeter based on the CIELAB system. Lightness (L*), red (+a*) to green (-a*), and green (+b*) to yellow (-b*) were measured using a colorimeter standardized against a white plate. Browning index (Cefola, et al., 2012), total color difference (ΔE), hue angle, and chroma (Lee, Seo, Rhee, & Kim, 2016) were calculated according to published methods according to the calculations are shown below.

Browning Index = 100(x-0.31)/0.17; [a_0* is the initial a* value; x = (a* + 1.75L*)/(5.645L* + a_0* –3.01b*)]

Hue angle = arctan (b*/a*)

Total color difference (ΔE) = $[(L_o^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2]^2$

Chroma = $[a^{*2} + b^{*2}]^2$

PPO was determined according to a published method (Soliva-Fortuny, Biosca-Biosca, Grigelmo-Miguel, & Martin-Belloso, 2002). Enzyme activity in crude PPO extract was assayed spectrophotometrically by adding 0.4 of 0.2 M catechol (Sigma-Aldrich) and 100 µL of extract to 5.5 mL phosphate buffer in a quartz cuvette of 1 cm path length. The changes in absorbance at 420 nm were recorded every 15s for 10 minutes from the time the enzyme extract was added using a Spectronic Genesys 5 (Thermo Electric Corporation, Madison, WI). PPO activity was defined as the slope of the time course of catechol oxidation, i.e., the change in absorbance of per min per mg of pawpaw pulp.

The measurement of total phenolics was determined spectrophotometrically by using the Folin–Ciocalteu (FC) assay as reported previously (Harris & Brannan, 2009). FC reagent was diluted with deionized water and the diluted reagent (750 µl) was mixed with aliquots of the pawpaw pulp extract (100 µl) and 7.5% bicarbonate solution (750 µl). After 120 minutes in the absence of light, absorbance was measured at 750 nm by using a Spectronic Genesys 5 (Thermo Electric Corporation, Madison, WI). Total phenolics were quantified according to a standard curve prepared from gallic acid and expressed as µmol gallic acid equivalents.

Total flavonoids were measured spectrophotometrically. Pawpaw extract (0.5 ml) was mixed with methanol (1.5 ml), to which 10% aluminum chloride (0.1 ml), 1 mol/l potassium acetate (0.1 ml), and deionized water (2.8 ml) was added. Samples were vortexed and allowed to sit for 40 minutes at 25 ℃, after which the absorbance was measured at 415 nm using a Spectronic Genesys 5 (Thermo Electric Corporation, Madison, WI). The total flavonoids contents were quantified by comparing to the standard curve prepared from rutin (quercetin-3-O-rutinoside) and the concentrations of total flavonoids were reported as µmol rutin equivalents (Bor, Chen, & Yen, 2006).

2.4 Statistical Analysis

Groups were analyzed using PASW Statistics 18 for Windows (Armonk, NY). Analysis of variance (ANOVA) was used to determine differences between the means. Significance was set at $p < 0.05$, and a post-hoc test, Duncan's Multiple Range test, determined where significant differences occurred.

3. Results and Discussion

3.1 Polyphenol Oxidase (PPO) Activity Pawpaw Pulp

PPO activity values for pawpaw pulp were measured for the main effect of month of storage at three levels (0, 4, 8 months) and the main effect of four levels of treatment. No significant difference in PPO activity was observed over the storage time variable ($p=0.195$), however, a significant effect of treatment was observed in PPO activity ($p<0.001$). *Post hoc* analysis revealed no significant difference between samples that were vacuum packaged and those for which no attempt to exclude air was made. The addition of the two chemical browning inhibitors significantly lowered PPO activity, with ascorbic acid exhibiting a significant 69% reduction in PPO activity compared to vacuum and air samples. NAC was significantly more effective than ascorbic acid and almost completely inhibited PPO activity compared to the vacuum and air samples.

Table 1 shows the mean values of PPO activity for the two-way interactions between storage time and packaging. For the samples for which air was not excluded, PPO activity remained constant during storage. Vacuum packaging reduced PPO activity by 29% after 8 months of storage. Addition of ascorbic acid to pawpaw pulp reduced PPO activity by 86% at 8 months of frozen storage, at which time PPO activity in the NAC-treated pulp was completely inhibited. Significantly lower levels of PPO were observed initially with the addition of the chemical inhibitors.

Table 1. Polyphenol oxidase activity (ΔABS/min/mg pawpaw pulp) of pawpaw pulp stored frozen for 8 months without air exclusion (Air), vacuum packaged (Vacuum), or with the addition of 1% (w/w) ascorbic acid or N-acetyl cysteine. Different superscripts denote significant differences ($p<0.05$)

Month of Storage	Air	Vacuum	Ascorbic Acid	N-Acetyl Cysteine
0	2.56 ± 0.11^{bc}	3.59 ± 0.18^{a}	1.20 ± 0.30^{d}	0.00 ± 0.00^{g}
4	2.83 ± 0.06^{b}	2.71 ± 0.43^{b}	1.06 ± 0.17^{e}	0.06 ± 0.06^{fg}
8	2.23 ± 0.004^{c}	2.55 ± 0.08^{bc}	0.17 ± 0.29^{f}	0.00 ± 0.00^{g}

3.2 Total Phenolics and Flavonoids of Pawpaw Pulp

The main effect of time of frozen storage (three levels: 0, 4, 8 months) and treatment (four levels: vacuum, air, ascorbic acid, NAC) on total phenolics were analyzed and were not significantly affected by either. Significant differences were observed for the two-way interactions between storage time and packaging for total phenolics (Table 2). Pawpaw pulp to which ascorbic acid or NAC exhibited no decline in total phenolics during storage. The mean value of total phenolics decreased by 41% in vacuum packed pawpaw pulp over 8 months of frozen storage and by 57% while stored for 8 months in the presence of air. The phenolic concentrations of pawpaw from this study are comparable to other fruits in the Annonacea family, including the soursop/guanabana/graviola, sugar apple, and cherimoya which ranged from 4-8 μmol gallic acid equivalents/g pulp (Almeida, et al., 2011; Barreca, et al., 2011).

Table 2. Total phenolic concentration (μmol gallic acid equivalents/g pawpaw pulp) of pawpaw pulp stored frozen for 8 months without air exclusion (Air), vacuum packaged (Vacuum), or with the addition of 1% (w/w) ascorbic acid or N-acetyl cysteine. Different superscripts denote significant differences ($p<0.05$)

Month of Storage	Air	Vacuum	Ascorbic Acid	N-Acetyl Cysteine
0	15.32 ± 1.38^{a}	9.78 ± 0.26^{bcd}	8.57 ± 0.17^{cde}	8.46 ± 0.63^{cde}
4	7.19 ± 0.15^{defg}	8.30 ± 0.73^{cde}	10.22 ± 0.09^{bc}	6.84 ± 0.41^{efg}
8	6.51 ± 0.50^{efg}	5.74 ± 1.07^{efg}	9.36 ± 0.96^{bcd}	7.71 ± 0.53^{cdef}

The main effects of time of frozen storage (three levels: 0, 4, 8 months) and treatment (four levels: vacuum, air, ascorbic acid, NAC) on total flavonoids were analyzed. Flavonoids were not significantly affected by storage time but were significantly affected by treatment ($p < 0.001$), with pawpaw treated with ascorbic acid and NAC exhibiting significantly lower flavonoids than the vacuum or air pulp. Previous research on vacuum packaged, frozen stored pawpaw pulp agrees with this finding, as flavonoid values initially increased then remained constant during 6 months of frozen storage (Harris & Brannan, 2009).

Table 3. Total flavonoid concentration (μmol rutin equivalents/g pawpaw pulp) of pawpaw pulp stored frozen for 8 months without air exclusion (Air), vacuum packaged (Vacuum), or with the addition of 1% (w/w) ascorbic acid or N-acetyl cysteine. Different superscripts denote significant differences ($p<0.05$)

Month of Storage	Air	Vacuum	Ascorbic Acid	N-Acetyl Cysteine
0	4.75 ± 0.21^{a}	1.36 ± 0.11^{def}	0.45 ± 0.61^{f}	1.52 ± 0.72^{def}
4	2.26 ± 0.10^{cde}	2.52 ± 0.08^{bc}	0.40 ± 0.34^{f}	0.86 ± 0.51^{ef}
8	1.75 ± 0.34^{def}	1.99 ± 0.21^{cdef}	0.61 ± 0.77^{ef}	1.03 ± 0.60^{def}

Research has shown that the effect of frozen storage on the level of antioxidant compounds in tropical fruit pulp is species dependent. For example, in the Brazilian tree fruit cambuci (*Campomanesia phaea*), total phenolic content was reduced by 28% in frozen fruit compared to fresh (Genovese, Pinto, Goncalves, & Lajolo, 2008). However, the reverse was true in guava (*Psidium guajava*), in which a 60% higher antioxidant content was observed in frozen-stored guava pulp compared to fresh guava pulp (Hassimotto, Genovese, & Lajolo, 2005). In pawpaws, pulp stored frozen for 300 days exhibited four times more total phenolics and flavonoids than fresh pulp (Harris & Brannan, 2009). The results reported here suggest that the oxidative degradation of total phenolics (Table 2) but not flavonoids (Table 3) in pawpaw pulp was enhanced by the presence of air during 8 months of frozen storage. This could mean that the flavonoid fraction, which is a subset of the total phenolic fraction, is not as susceptible to air-induced oxidation as other phenolic compounds. One possible reason for the degradation of phenolic compounds in pawpaw pulp could be the activity of PPO, which was inhibited in pawpaw pulp to which ascorbic acid and NAC were added (Table 1). Research has shown that PPO can catalyze oxidative reactions that use phenolic compounds as substrates, causing a significant decrease of total phenolics (De Leonardis & Macciola, 2011). Research also has shown that PPO can cause the oxidative degradation of flavonoids such as catechol, chlorogenic acids (Kader, Nicolas, & Metche, 1999), and quercetin (Makris & Rossiter, 2000). Because chlorogenic acids have been identified in pawpaw pulp (Brannan, Peters, & Talcott, 2015), they potentially could serve as a substrate for polymerization of phenolics, which could lead to the decreased level of total phenolics detected by the Folin–Ciocalteu assay. However, polyphenol oxidase is not the only enzyme that can cause polymerization. Perhaps there are other enzymes that could catalyze polymerization or other reactions that consume other pawpaw pulp phenolics as substrates. More research on enzymatic degradation would be required in order to further investigate the cause of total phenolic loss.

It should be noted that the addition of ascorbic acid and NAC to pawpaw pulp inhibited PPO activity. Thus, both ascorbic acid and NAC could protect flavonoids. However, the results show that the level of flavonoids in pawpaw pulp treated with ascorbic acid and NAC is significantly less than that detected in the air packaging and vacuum packaging samples. This suggests oxidation or polymerization of flavonoids, if occurring, is due to another mechanism.

3.2 Color of Frozen-stored Pawpaw Pulp

CIELAB color values (L*, a*, b*) were used to generate the applied color values total color difference (ΔE), browning index, hue and chroma in pawpaw pulp for the two main effects of frozen storage at three levels (0, 4, 8 months) and treatment at four levels (vacuum, air, ascorbic acid or NAC). The applied values for browning index, total color difference, hue, and chroma often are used in the literature to characterize the color or browning of specific commodities. With the exception of hue, which we utilized in a previous paper (Brannan, Peters, & Talcott, 2015), this is the first study to utilize the suitability of these indices for pawpaw. Analysis of variance for the main effects showed significance for all seven color attributes at $p<0.001$. For the main effect of storage time, ANOVA showed significance during storage for all seven color attributes at $p<0.001$, indicating that there were color changes during storage. Pawpaw pulp samples at 8 months of storage were significantly darker (lower L*), more yellow (higher b*), more vivid (higher chroma), and had a higher browning index than the samples at 0 or 4 months of storage. For the main effect of treatment, ascorbic acid and NAC treatment produced pawpaw pulp that was significantly different than samples to which air was not excluded for all seven dependent color variables. Specifically, NAC and ascorbic acid produced pulp that was lighter (higher L*), less red (lower a*), and more yellow (higher b* and hue), more vivid (higher chroma), and exhibited more color difference (higher ΔE).

Table 4 shows the color values of pawpaw pulp for the two-way interactions of storage time and treatment. The color of pawpaw pulp that was stored without air exclusion during frozen storage changed as indicated by the significant differences observed during storage time for L*, a*, b*, browning index, and chroma. Pawpaw pulp stored for 8 months without air exclusion was significantly darker (lower L*), redder (higher a*), and less yellow (lower b*) than the three other treatments. This agrees with a previous study of sensory analysis of pawpaw (Brannan, Salabak, & Holben, 2012) that compared samples with and without vacuum packaging. The treatments – vacuum packaging and the addition of ascorbic acid or NAC – mitigated the color change during frozen storage. Differences after 8 months of storage between the air-stored samples and the vacuum packaged and chemically-treated samples also were observed for hue and chroma but not for browning index or ΔE. Specifically, significantly lower hue and chroma values were observed in the air-exposed pawpaw pulp that was not vacuum packaged or treated with ascorbic acid or NAC, indicating that it was a dull red/yellow color, probably perceived as brown, as opposed to vivid yellow of the treated samples. Both chemical treatments, ascorbic acid and NAC, were very effective at mitigating color changes during the frozen storage period.

Table 4. CIELAB color values (L*, a*, b*), total dolor difference (ΔE), browning index (B.I.), hue and chroma of pawpaw pulp stored frozen for 12 months without air exclusion (Air), vacuum packaged (Vacuum), or with the addition of 1% (w/w) ascorbic acid or N-acetyl cysteine (NAC). Means within an attribute/treatment combination with different superscript letters are significantly different at $p<0.05$

		Months of Frozen Storage		
Attribute	Treatment	0	4	8
L*	Air	52.1 ±0.4^{g}	58.5 ±1.2^{e}	54.3 ±1.0^{f}
	Vacuum	65.8 ±0.6^{bc}	61.7 ±0.2^{d}	61.7 ±0.5^{d}
	Ascorbic Acid	70.7 ±0.5^{a}	66.3 ±0.6^{b}	65.7 ±0.5^{bc}
	NAC	66.6 ±0.4^{b}	65.0 ±1.5^{c}	67.0 ±0.4^{b}
a*	Air	6.9 ±0.3^{b}	6.9 ±1.8^{b}	10.3 ±0.2^{a}
	Vacuum	5.9 ±0.2^{bc}	6.2 ±1.0^{bc}	6.7 ±0.4^{b}
	Ascorbic Acid	7.1 ±0.7^{b}	5.1 ±0.6^{c}	5.0 ±0.7^{c}
	NAC	5.9 ±0.2^{bc}	3.6 ±0.3^{d}	5.0 ±0.2^{c}
b*	Air	19.3 ±2.0^{g}	25.8 ±1.6^{f}	27.3 ±0.6^{ef}
	Vacuum	29.3 ±0.1^{e}	29.0 ±1.7^{ef}	37.3 ±0.7^{bcd}
	Ascorbic Acid	44.1 ±2.3^{a}	36.8 ±2.2^{cd}	37.4 ±3.1^{bcd}
	NAC	40.2 ±0.3^{bc}	36.0 ±3.4^{d}	40.6 ±0.6^{b}
ΔE	Air	N.D.[1]	9.2 ±1.9^{a}	9.0 ±0.7^{a}
	Vacuum	N.D.[1]	4.3 ±0.5^{b}	9.0 ±0.7^{a}
	Ascorbic Acid	N.D.[1]	8.9 ±1.8^{a}	8.8 ±2.9^{a}
	NAC	N.D.[1]	5.2 ±3.2^{b}	1.2 ±0.5^{c}
B.I.	Air	54.4 ±6.1^{f}	64.7 ±6.7^{e}	84.6 ±1.0^{bc}
	Vacuum	63.3 ±0.4^{ef}	68.6 ±6.5^{de}	95.1 ±4.2^{a}
	Ascorbic Acid	98.2 ±6.8^{a}	80.0 ±6.9^{c}	82.7 ±9.5^{bc}
	NAC	92.6 ±2.2^{ab}	77.9 ±7.1^{cd}	91.1 ±1.5^{ab}
Hue	Air	70.2 ±1.1^{h}	75.1 ±2.8^{g}	69.2 ±0.7^{h}
	Vacuum	78.6 ±0.3^{ef}	77.9 ±1.1^{f}	79.9 ±0.4^{de}
	Ascorbic Acid	80.8 ±1.0^{cd}	82.1 ±1.0^{bc}	82.4 ±0.5^{abc}
	NAC	81.6 ±0.2^{bcd}	84.2 ±1.0^{a}	83.0 ±0.3^{ab}
Chroma	Air	20.8 ±2.0^{g}	26.8 ±2.0^{f}	29.2 ±0.5^{f}
	Vacuum	29.9 ±0.1^{f}	29.7 ±1.8^{f}	37.8 ±0.8^{bcd}
	Ascorbic Acid	44.7 ±2.2^{a}	37.1 ±2.2^{de}	37.7 ±3.1^{bcd}
	NAC	40.6 ±0.4^{bc}	36.2 ±3.4^{d}	40.9 ±0.6^{b}

[1]N.D. Total color difference calculated based on the difference from initial storage.

3.3 Correlations between PPO, Phenolics, Flavonoids, and Color of Pawpaw Pulp

Because PPO is the enzyme responsible for pawpaw browning and phenolics are the substrate for PPO, correlations among PPO activity, total phenolics, flavonoids, and the color attributes were analyzed (Table 5). PPO activity was negatively correlated with b*, browning index, hue, and chroma. This suggests that the color changes associated with a decline in PPO activity are associated with increased yellowness, vividness, and overall browning. From the perspective of PPO substrates, total phenolics only were correlated with browning index whereas total flavonoids were strongly correlated negatively with L*, b*, browning index, hue and chroma. PPO activity was not significantly correlated with either total phenolics or flavonoids (data not shown).

Table 5. Pearson's correlation coefficient (*r*) and significance level (P-value in parentheses) of pawpaw pulp for CIE tristimulus color values (L*, a*, b*) and applied color values total color difference (ΔE), browning index, hue, and chroma

	L*	a*	b*	ΔE	Browning Index	Hue	Chroma
Polyphenol Oxidase activity	ns[1]	ns	*r*=-0.700 (0.011)	ns	*r* = -0.581 (0.047)	*r*=-0.656 (0.021)	*r* = -0.697 (0.012)
Total Phenolics	ns	ns	ns	ns	*r* = -0.589 (0.044)	ns	ns
Total Flavonoids	*r*=-0.819 (0.001)	ns	*r*=-0.815 (0.001)	ns	*r* = -0.655 (0.021)	*r*=-0.733 (0.007)	*r* = -0.812 (0.001)

[1]ns = not significant

There are many ways to inhibit enzymatic browning. Heat treatment to denature PPO is not an option in fruit pulp. Chemical inhibitors can directly deactivate PPO, react with intermediates of the enzymatic browning reaction, or modify or remove substrates or cofactors (Pilizota & Subaric, 1998). It has long been known that sulfites are a potent enzymatic browning inhibitor that change PPO structure and therefore irreversibly inhibit PPO activity (Sayavedrasoto & Montgomery, 1986). However, the FDA prohibited the use of sulfites on fruits because of their toxicity so they are not an option to inhibit enzymatic browning in pawpaw pulp (Timbo, Koehler, Wolyniak, & Klontz, 2004), which led to alternatives such as ascorbic acid or thiol-containing compounds (Pilizota & Subaric, 1998).

The results of this study indicate that ascorbic acid inhibited PPO activity but not browning during frozen storage of pawpaw pulp. Ascorbic acid is reported to reduce PPO activity by chelating a copper atom from the prosthetic group of PPO or reducing quinones back to phenols (Limbo & Piergiovanni, 2006). Fruits from the Annonaceae family naturally are rich in ascorbic acid. Pawpaw pulp contains 4.98 mg ascorbic acid per 100 g (Harris & Brannan, 2009) but it is reported that endogenous ascorbic acid does not completely inhibit PPO (Fang, Wang, Xiong, & Pomper, 2007).

NAC inhibited PPO activity and prevented browning. The mechanism of NAC inhibition of PPO has been speculated to be that it reacts with quinones at the initial stage of the enzymatic browning reaction to reduce O-quinones to O-diphenols, producing colorless products (Demirkol, Adams, & Ercal, 2004). Naturally-occurring NAC is found in fruits such as lemon, grape, and strawberry in the range of 4-5 nM/g wet weight (Demirkol, Adams, & Ercal, 2004), but NAC content has not been measured in pawpaw. In pears, NAC has been shown to be more effective in combination with 1.5% glutathione rather than alone (Oms-Oliu, Aguilo-Aguayo, & Martin-Belloso, 2006). This does not appear to be the case in pawpaw, as NAC completely inhibited PPO and prevented browning.

4. Summary and Conclusion

Enzymatic browning could have a significant effect on both food quality and food nutrition value. Enzymatic browning in pawpaw pulp produces a color deemed undesirable. Although commercial frozen pawpaw pulp preserved with ascorbic acid is on the market, anecdotal evidence suggests that this pulp browns during storage and especially quickly once thawed. A strategy to inhibit enzymatic browning during frozen storage would be useful for the nascent pawpaw industry.

The negative correlations found between flavonoids and L*, b*, browning index, hue, and chroma indicate that PPO-induced enzymatic browning could reduce the overall antioxidant content of the pulp by consuming flavonoids. Recent research has shown that flavonoids identified from pawpaw pulp are largely flavan-3-ols, particularly (-)-epicatechin, B-type procyanidin dimers, and procyanidin trimers. Other foods containing procyanidins include cocoa, tea, wine, and berries, and these foods have become well-known for their potential health benefits and antioxidant activity. This could be the basis for a value-added fresh or frozen pawpaw pulp industry if the browning of pawpaw pulp can be controlled with chemicals such as ascorbic acid or NAC. Other substances with similar phytochemical profiles to pawpaw, such as grape seed extract (Weber, et al., 2007), have been commercialized as powerful antioxidant food additives, which could be another vehicle to pawpaw commercialization.

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