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Loss of Ripening Capacity of Pawpaw Fruit with Extended Cold Storage

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The fruit ripening traits of pawpaw [*Asimina triloba* (L.) Dunal] were examined after harvest and after cold storage at −2, 2, 4, and 6 °C for up to 12 weeks. Generally, fruits stored at 2−4 °C for 4 weeks ripened normally, but those stored at −2 °C did not ripen normally, those stored at 6 °C were overripe, and by 6−8 weeks those stored at 2−4 °C had a lower respiration rate and ethylene production, lower firmness, and lower pH than fruit cold-stored for 4 weeks or less. These changes, and the occasional development of brown discoloration in the pulp once the fruits were moved back to room temperature, were evidence of chilling injury by 6 weeks. After harvest and through 4 weeks of cold storage, the main volatile compounds produced by fruit were methyl and ethyl octanoates and hexanoates. Volatile production significantly increased >5-fold in fruit ripened for 72 h after harvest or after removal from up to 4 weeks of cold storage. Fruit cold-stored for 6 weeks or more produced fewer total volatiles and esters but increased levels of such off-flavor compounds as ethyl acetate, ethyl propionate, and hexanoic and decanoic acids. Alcohol acyltransferase (AAT) activity declined in cold-stored fruit but was not correlated with either total volatile production or total ester production. Alcohol dehydrogenase activity did not change during ripening after harvest or cold storage. Lipoxygenase activity was highest just after harvest or after 2 weeks of cold storage, but was low by 4 weeks. Thus, ripening of pawpaw fruit seems to be limited to 4 weeks at 2−4 °C with loss of ability to continue ripening and chilling injury symptoms evident at colder temperatures and after longer periods of cold storage.

KEYWORDS: Regular atmosphere storage; ethylene; respiration; firmness; sugar; aroma volatile; alcohol acyltransferase; alcohol dehydrogenase; lipoxygenase

INTRODUCTION

Pawpaw [*Asimina triloba* (L.) Dunal] fruits are climacteric with both respiratory and ethylene peaks detected within 3 days after harvest (*1−3*). During this same period, fruits soften rapidly at ambient temperature. The firmest fruits at harvest are too soft for handling within 5 days. Due to the rapid decline in fruit firmness, pawpaw fruits are extremely perishable, a characteristic that they share with other members of the Annonaceae family such as cherimoya (*4*), and this presents a significant challenge in postharvest handling.

Pawpaw fruits harvested when they are just beginning to soften have been stored for 1 month at 4 °C with little loss in quality (*1*). Cold storage delayed the ripening process of both ripe and unripe fruits. However, upon removal from cold storage, firmness declined rapidly, accompanied by climacteric increases in ethylene production and respiration. For cold-stored fruit the ethylene peak was higher than that of fruit immediately after harvest, and it was detected within 4 days upon removal from storage. Preliminary observations indicated that longer storage periods resulted in external and internal brown-to-black discoloration of the fruit tissues (*5*), possibly symptoms of chilling injury similar to that found with other Annonaceae such as cherimoya (*6*).

Cold-storage injury symptoms in fruit are a response of many species to temperatures at or near freezing and of many tropical and subtropical species to nonfreezing temperatures below 10−12 °C, often termed chilling injury (*7*). External symptoms of chilling injury include scald or skin discoloration, skin desiccation, pitting, internal breakdown, uneven ripening, development of large sunken areas, poor flavor, and poor color development. Physiological responses may include low ethylene production, impaired photosynthesis, accumulation of acetaldehyde, ethanol, and other toxic compounds, activation of proteolytic enzymes, and programmed cell death after prolonged cold storage (*8*).

Two of the most distinctive traits of pawpaw fruit are the aroma and flavor (*9*). The aroma of tree- or postharvest-ripened pawpaw fruit was due to the high concentration of methyl and ethyl esters in the fruit (*10, 11*). Retention of this trait after

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cold storage would be critical to successful marketing. However, it is not known how cold storage may affect volatile biosynthesis or production by pawpaw fruit after removal from cold storage.

Other than the observation that chilling injury may occur in pawpaw fruit cold-stored for >4 weeks, little is known about the effect of extended periods of cold storage on poststorage ripening and fruit quality traits. Hence, the aim of this study was to investigate the effects that cold storage temperature and length have on selected ripening and fruit quality parameters including respiration, ethylene production, firmness, sugar content, titratable acidity, and aroma volatile production.

MATERIALS AND METHODS

Fruit Harvest and Cold Storage. Pawpaw fruits exhibiting some tissue softening as determined by touch were harvested from the Kentucky State University Research Farm, Frankfort, KY, on several dates during the August−October harvest seasons of 2001, 2002, 2004, and 2005. Immediately following harvest, fruits were transported to the laboratory at the University of Kentucky, Lexington, KY. During cold storage individual fruits were sealed in poly bags (permeable to CO_2, O_2, and C_2H_4), and ethylene traps were added to the cooler.

In 2001 and 2002, fruits from several genotypes were pooled for cold storage and subsequent analyses. Fruits were stored at −2, 2, and 6 °C for 0, 1, 2, 4, 8, and 12 (2001 only) weeks. There were at least 10 fruits per storage temperature and duration.

In 2004 and 2005, fruits were harvested from the cultivars 'Wabash', 'Middletown', 'PA Golden', 'Taylor', 'Taytwo', 'Shenandoah', 'Wells', and 'Wilson' and advanced selections from the PawPaw Foundation breeding program, '8-20' and '9-58'. In addition, unripe fruits were collected from 'Taytwo' 1 week before normal harvest in 2005. On each harvest date pawpaw fruits were placed in 4 °C storage for 0, 2, 4, 6, or 8 weeks. For each storage period, there were 15 fruits/cultivar in 2004 and 10 fruits/cultivar in 2005.

Upon harvest or removal from storage fruits were moved to ambient temperature (21 ± 2 °C) and ripened on a laboratory bench. Fruit weight, firmness, respiration rate, and ethylene production were measured on five randomly selected fruits daily for 5 days in 2001 and 2002, daily for 3 days in 2004, and on days 1 and 3 in 2005. After these measurements, the fruits were peeled, sectioned, and individually frozen in −80 °C storage.

Respiration and Ethylene Production. Rates of respiratory CO_2 and C_2H_4 production by individual fruits were obtained by analyzing headspace composition of fruits held for 2 h in enclosed bottles (*1*), starting at 4 or 72 h after harvest or removal from cold storage.

Firmness. The external firmness of each fruit was measured after respiration and ethylene production measurements by compression with a Chatillon Force Gauge (*1*).

HPLC Sugar Analysis. High-performance liquid chromatography using pulsed electrochemical detection (HPLC-PED) analysis was performed on three replicate extractions from fruits of '8-20' harvested in 2004 at the ripe stage. As described by Holland et al. (*12*), 200 mg of thawed fruit pulp was extracted with 1 mL of 80% ethanol, the supernatant was collected, the pellet was re-extracted two more times, and the combined supernatants were used to determine soluble sugars. The supernatant was dried and redissolved in 1 mL of Millipore water. The reconstituted aqueous solution was filtered through 0.45 μm nylon filters (Costar Spin-X, Corning Inc., Corning, NY) and diluted 1000× to be within the linear range of HPLC sensitivity. Sugars were quantified using a Dionex BioLC system (Dionex Corp., Sunnyvale, CA), with degassed NaOH (200 mM) and degassed water as mobile phase (9−91%, v/v), a sample loop volume of 25 μL, and on a 4 × 250 mm CarboPac PA1 Analytical (P/N 46110) column at a flow rate of 1.0 mL/min. Quantitative determinations were made by comparing the sample results with an external standard mixture of sucrose, glucose, and fructose. Pawpaw sugars were identified by their retention times. 2-Deoxyglucose was used as an internal standard from which calculations of percent recovery were estimated. The major sugars identified, sucrose, glucose, and fructose, were determined as milligrams per gram of fresh weight (FW).

Soluble Sugar and Starch Quantification. Soluble sugar and starch contents were quantified from 'Taytwo' fruit harvested in 2005 that were unripe (1 week before the start of harvest), or at harvest, or after 2, 4, 6, or 8 weeks of cold storage followed by 4 and 72 h of ripening, following extraction as above. For glucose analysis, 20 μL of the reconstituted soluble sugar fraction was added to 80 μL of water. For sucrose analysis, 20 μL of the soluble sugar fraction was added to 480 μL of Tris buffer (10 mM, pH 4.5), and 50 μL of invertase (10 mg of invertase/mL Tris buffer), previously desalted through a Sephadex G-50 column, was then added. The mixture was vortexed and incubated in a water bath at 55 °C overnight.

The pellet remaining after soluble sugar extraction was used for starch determination. To 5 mg of washed pellet was added 1 mL of sodium acetate buffer (100 mM, pH 4.5). The mixture was vortexed, boiled for 1 h, and cooled to room temperature. Then, 50 μL of desalted amyloglucosidase (0.1 g in 1 mL of sodium acetate buffer) (Sigma, St. Louis, MO) was added to each sample, and the mixture was vortexed, incubated in a water bath at 55 °C overnight, and centrifuged for 5 min at 14000*g*. The supernatant was diluted 100-fold with distilled water for analysis of the glucose released from starch.

Glucose in each resulting fraction above was quantified using the peroxidase−glucose oxidase (PGO) enzyme color reagent kit (Sigma, St. Louis, MO). To 0.1 mL of diluted supernatant, invertase-treated supernatant, or amyloglucosidase-treated pellet solution was added 1 mL of PGO reagent. The resulting solution was incubated at 37 °C for 30 min. Absorbance was read at 450 nm, and a glucose standard curve was used for quantification. Free glucose in pawpaw tissue extracts and glucose released from sucrose and from starch were determined.

Titratable Acidity (TA) and pH. TA was determined by mixing 10 g of 'Taytwo' pawpaw pulp with 20 mL of distilled water. The homogenate was filtered, the initial pH recorded, and the solution titrated with 0.1 M NaOH to pH 8.1 as described by Polenta et al. (*13*). Results were expressed as millimoles of malic acid equivalents per 100 g of FW because, as reported by Maldonado et al. (*14*), this is the predominant organic acid in cherimoya, a species related to pawpaw fruit.

Volatile Measurement. The volatile profile was assayed for every cultivar at harvest and for 'Taytwo' and 'Wells' samples ripened for 72 h after removal from each cold storage period in 2005. Frozen tissue collected as described above was used for volatile measurement. Approximately 10 g tissue samples were analyzed according to methods described by Hamilton-Kemp et al. (*15*). Ethyl alcohol, ethyl acetate, ethyl butanoate, methyl hexanoate, ethyl hexanoate, methyl octanoate, ethyl octanoate, ethyl decanoate, ethyl propionate, and methyl butanoate were identified using mass spectrometric analysis, whereas hexanoic acid and octanoic acid were identified from retention times, each matching those of authentic standards. All standards were purchased from Sigma-Aldrich (St. Louis, MO) or were gifts from Bedoukian Chemical Co. (Danbury, CT). A semiquantitative analysis of total volatile production (or that adsorbed to the SPME fiber) of three fruits per sampling time was calculated by summing the area units (AU) of both identified and unknown volatiles with AU > 20 (an arbitrary cutoff point) and from the AU of the individual volatile compounds. AU values were converted to micrograms per gram of FW using a regression of ethyl hexanoate amount versus area units, this compound being the dominant volatile produced by pawpaw fruit. This assumes the same response factor among all compounds.

Alcohol Acyltransferase (AAT) Assay. Extraction and assay methods of AAT were based on those described by Ke et al. (*16*) and Defilippi et al. (*17*). For each replicate, 6 g of 'Taytwo' pawpaw pulp tissue was homogenized in a Polytron homogenizer in 18 mL of 100 mM potassium phosphate buffer (pH 7.5) containing 2 g of polyvinylpolypyrrolidone (PVPP). The AAT activity assay was performed by adding 750 μL of potassium phosphate buffer (100 mM, pH 7.5), 10 μL of 1 M $MgCl_2$, 100 μL of 10 mM 5,5′-dithiobisnitrobenzoic acid (DTNB), 20 μL of 20 mM isoamyl alcohol, and 20 μL of 50 mM acetyl CoA. The assay solution was gently mixed in a cuvette, and 100 μL of fruit extract was added to a total volume of 1 mL. The increase in absorbance at 421 nm over time was measured spectrophotometrically (model Cary 50 Bio, Varian Analytical Instruments, Walnut Creek, CA) to follow the production of the yellow thiophenol

product (2-nitro-5-thiobenzoic acid) formed by the reaction of DTNB with free CoA released from the AAT esterification reaction. One activity unit was defined as the increase in 1 unit of absorbance per minute, and results were expressed as milliunits per milligram of protein. Protein was determined according to the method of Bradford (*18*) using BSA as a standard.

Alcohol Dehydrogenase (ADH) Assay. On the basis of methods for ADH assay in Defilippi et al. (*19*), 3 g of 'Taytwo' pawpaw pulp tissue of each replicate was homogenized in a Polytron homogenizer in 10 mL of 100 mM 2-(*N*-morpholino)ethanesulfonic acid (MES) buffer (pH 6.5) containing 2 mM dithiothreitol and 1% (w/v) PVPP. The homogenate was filtered through four layers of cheesecloth and centrifuged at 27000*g* for 15 min at 4 °C. The supernatant was recovered, passed through a Sephadex G50 column, and used for the enzyme assay. The reduction of acetaldehyde was followed spectrophotometrically at 25 °C by measuring the change in absorbance at 340 nm for 2 min of a reaction mixture containing 800 μL of MES buffer (100 mM, pH 6.5), 50 μL of 1.6 mM NADH, 100 μL of enzyme extract, and 50 μL of 80 mM acetaldehyde. The reaction was initiated by the addition of acetaldehyde. One activity unit was defined as the increase in 1 unit of absorbance per minute, and results were expressed as milliunits per milligram of protein. Protein was determined as above.

Lipoxygenase (LOX) Assay. LOX extraction and activity were measured by modifying the methodology used by Myung et al. (*20*). For each replicate, 3 g of 'Taytwo' pawpaw pulp tissue was homogenized in a Polytron homogenizer in 10 mL of 1 M Tris HCl buffer (pH 8.5), containing 15% sucrose, 10% (w/v) Triton X-100, 0.5% (w/v) PVPP, 1 M KCl, 10 mM $MgCl_2$, and 1:50 protease inhibitor cocktail [Plant Fractionated Protein Extraction Kit (Sigma)]. LOX activity was assayed spectrophotometrically by mixing 980 μL of assay solution [40 mM sodium acetate buffer, pH 4.5, containing 1 mM linolenic acid (10% ethanol solution), 0.14% (v/v) Tween-20, and 1 M KOH] with 20 μL of enzyme extract. The activity was followed by measuring the increase in absorbance at 235 nm due to the formation of hydroperoxides from linolenic acid for 1 min. One activity unit was defined as the increase in 1 unit of absorbance per minute, and results were expressed as milliunits per milligram of protein. Protein was determined as above.

Statistical Analysis. Preliminary analyses of the data indicated that there were no differences among genotypes or cultivars in absolute values or patterns of respiration rate, ethylene production, fruit firmness, or visual evidence of chilling injury after the storage periods (data not shown). Thus, the data were pooled across genotypes for statistical analyses and presentation. All data were subjected to analysis of variance using SAS version 9.1 software (SAS Institute Inc., Cary, NC). Means were compared by Fisher's protected least significance difference (LSD, $P = 0.05$), and single degree of freedom contrasts were used to compare 4 versus 72 h values and to determine if there were linear or quadratic trends across storage periods.

RESULTS AND DISCUSSION

Fruit Firmness, Respiration, and Ethylene Production. In 2001, pawpaw fruits remained firm enough for handling upon removal from cold storage at all storage temperatures through 8 weeks (**Figure 1A**). At 12 weeks, fruits at all storage temperatures exhibited an apparent increase in firmness, although this was likely due to the increasing leatheriness of the peel and slight shriveling of the fruit. The general decline in firmness during cold storage at 2 and 6 °C indicated that fruit softening continued at these temperatures. Archbold and Pomper (*1*) observed little firmness loss by fully ripe fruit (firmness ~ 6 N) during cold storage for 4 weeks.

Mean respiration generally showed an increase through 2 weeks of 2 °C and 4 weeks of −2 °C storage, whereas fruits stored at 6 °C showed a decline (**Figure 1B**). Beyond 4 weeks, fruits from all storage temperatures exhibited declining respiration. Mean ethylene production of fruits stored at 2 °C exhibited an increase through 4 weeks of cold storage and low rates at 8 and 12 weeks, whereas those stored at −2 and 6 °C showed little change through 12 weeks (**Figure 1C**). Maximum respiration and ethylene production at 72 h after harvest was 174 mg of CO_2/kg of FW h and 2.7 μg of C_2H_4/kg of FW h, respectively, and 250 mg of CO_2/kg of FW h and 44 μg of C_2H_4/kg h, respectively, at 72 h after removal from cold storage. The high respiration rate but low ethylene production of fruits held at −2 °C for 4 weeks and then moved to ambient temperature may indicate a chilling injury response. Elevated respiration rates have occurred in cold-sensitive species such as squash, tomato, and banana following removal from cold storage (*21*). Fruits held at 2 °C seemed to have the greatest capacity to ripen normally as shown by respiration and ethylene production through 4 weeks. The increasing ethylene production after removal from 2 °C storage through 4 weeks suggests that no irreparable damage occurred to the ethylene biosynthetic pathway. Fruits held at 6 °C may have ripened during cold storage as they exhibited a loss of firmness and low respiration and ethylene production rates by 1 week after the start of cold storage.

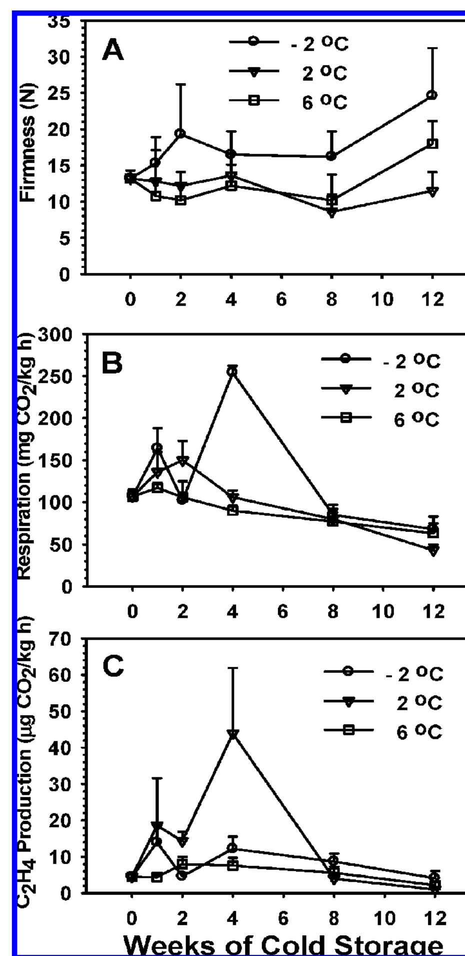

Figure 1. Mean firmness (**A**), respiration (**B**), and ethylene production (**C**) of pawpaw fruit measured daily for 3 days after harvest or after 2, 4, 6, 8, or 12 weeks of cold storage in 2001. Values are means + SE of 20 or more fruits.

In 2002, 2004, and 2005, fruits stored at 2−4 °C for up to 8 weeks exhibited very similar responses to those in 2001 (data not shown). However, an increasing problem with mold development in 2 °C storage was observed in 2004 and 2005. By 8 weeks, >80% of the fruits were affected, and these fruits were excluded from quality analyses. The University of

Table 1. Pulp Glucose, Sucrose, and Starch Content and pH of Unripe (Collected 1 Week before Harvest), Ripe (on the Day of Harvest), and Cold-Stored 'Taytwo' Pawpaw Fruit[a]

	glucose		sucrose		starch		pH	
cold storage (weeks)	4 h	72 h	4 h	72 h	4 h	72 h	4 h	72 h
	Content (Milligrams of Glucose per Gram of Fresh Weight)							
unripe	3		22		45		7.5	
0 (harvest)	3	16	20	86	31	21	6.5	7.4
2	8	19	42	89	31	8	7.2	7.2
4	11	14	80	94	8	24	6.4	7.2
6	19	19	55	94	9	6	6.5	7.2
8	38	29	100	59	10	8	5.3	5.2
	Contrasts (*P*)							
4 vs 72 h	0.8810		0.5330		0.9108		0.0053	
linear	0.1220	0.4313	0.0012	0.0014	0.0314	0.0403	<0.0001	<0.0001
quadratic	0.0019	0.0675	0.0005	0.9616	0.5091	0.8275	<0.0001	0.0044

[a] Fruits were cold-stored at 4 °C for 2, 4, 6, or 8 weeks and ripened for 4 or 72 h after harvest or removal from cold storage. Sucrose and starch contents were expressed as mg of glucose released/g of FW. Data are the average value of $n = 3$ measurements. Statistical analyses of 4 versus 72 h values and trends across storage time by single degree of freedom contrasts are shown.

Kentucky Plant Disease Diagnostic Laboratory identified *Cladosporium* spp. and *Fusarium* spp. as the major fungal organisms on the diseased pawpaw fruits.

Soluble Sugar and Starch Contents. For '8-20' fruit harvested in 2004, HPLC analysis showed that ripe fruit contained sucrose at 66 ± 6 mg/g of FW, fructose at 9 ± 2 mg/g of FW, and glucose at 5 ± 2 mg/g of FW. Small quantities of other sugars such as *myo*-inositol at 1.5 ± 0.3 mg/g of FW, galactinol at 0.02 ± 0.01 mg/g of FW, sorbitol at 0.2 ± 0.1 mg/g of FW, mannitol at 0.06 ± 0.03 mg/g of FW, and galactose at 0.23 ± 0.02 mg/g of FW were detected as well. Peterson et al. (*22*) reported that pawpaw fruit at harvest contained 60 mg/g of FW sucrose, 13 mg/g of FW fructose, and 18 mg/g of FW glucose, values comparable to those of the present work, whereas McGrath and Karahadian (*23*) reported 219 mg of total soluble solids/g of FW in ripe pawpaw.

'Taytwo' fruit sucrose content increased linearly across cold storage periods for both 4 and 72 h, reaching the highest concentrations by 8 or 4 weeks, respectively (**Table 1**). Glucose content increased quadratically across storage dates for the 4 h measurements only. Starch content of unripe fruit gradually decreased during ripening, as reported by McGrath and Karahadian (*23*), and was low in cold-stored fruit by 4 weeks. Whereas the loss of starch coincided with the increase in sucrose content, starch degradation accounted for only ~13% of the increase in sucrose and glucose during ripening after harvest. Thus, alternative sources of C are probably used for sucrose synthesis, although these C sources are unknown in pawpaw.

TA and pH. Fruits of the cultivar 'Taytwo' at harvest exhibited an average TA of 0.94 mmol of malic acid equiv/100 g of FW, values comparable to those reported for cherimoya (*14, 24*) but 5-fold lower than those for apple (*25*). In contrast to cold-stored cherimoya (*14*), pawpaw TA did not significantly change during ripening or cold storage (data not shown).

Fruit tissue had a pH of 6.54 at harvest, and it significantly increased from 4 to 72 h of ripening but decreased across all storage periods (**Table 1**). In cherimoya, malate accumulation in the cell was responsible for cytoplasmic acidification (*26*).

Pawpaw Volatile Profile during Ripening after Harvest. At harvest, when fruits were in the early stage of ripening, fruits produced several volatile compounds, although methyl octanoate was dominant, comprising 34−65% of the total volatile production among all 10 cultivars and advanced selections examined (data not shown). Interestingly, some individual fruits produced only small amounts of methyl octanoate with no other compounds evident (data not shown), suggesting it is the first volatile product indicative of ripening. Because octanoic acid represented >20% of the total pawpaw pulp fatty acid (*10, 27*) and octanoic acid was present at higher levels than hexanoic acid in the headspace (**Table 2**), the presence of volatile esters of this fatty acid may be explained by its abundance.

Total volatile production was >5-fold greater at 72 h of ripening than at 4 h (data not shown), so only the 72 h values are presented (**Table 2**). Total volatile production was greatest after 2 and 4 weeks of cold storage, resulting in a significant quadratic trend for total as well as all individual volatiles except ethyl alcohol across storage periods. The primary volatile compounds produced from harvest through 4 weeks of cold storage were ethyl octanoate, methyl octanoate, ethyl hexanoate, and methyl hexanoate (**Table 2**), comprising ≥79% of the total volatile profile after ripening for 72 h. Some of the same esters were observed in other Annonaceae such as soursop, cherimoya, and custard apple (*4, 28*) and in tropical species such as banana and mango (*29, 30*). The relative abundance of these types of volatiles produced by pawpaw may account for its similarity to the flavor of tropical species.

Once storage duration exceeded 4 weeks, the compounds indicative of chilling injury and/or altered volatile biosynthesis, including ethyl alcohol, ethyl acetate, ethyl propionate, octanoic acid, and hexanoic acid, significantly increased from 2% of total volatiles at harvest to 20% by 6 weeks and 52% by 8 weeks of cold storage, whereas methyl and ethyl hexanoates and octanoates declined to 31% after 8 weeks of cold storage. The increasing ethanol suggests that long-term exposure to cold caused mitochondrial impairment and a switch from aerobic to anaerobic metabolism as shown for cherimoya (*31*), avocado, and mango (*32*). However, the similarities among volatile profiles observed at harvest (0 weeks) and after 2 and 4 weeks of cold storage demonstrated that pawpaw fruit can be cold-stored for as long as 4 weeks without any major change in aroma quality. After 4 weeks of cold storage, the change in fruit volatile composition would likely affect consumer perception.

AAT Activity during Ripening and Cold Storage. In 'Taytwo' pawpaw, AAT activity was 85 milliunits/mg of protein at 72 h after harvest, and it declined across cold storage periods (**Figure 2**). There was no correlation between AAT activity and total volatile production ($r = 0.51$, $P > 0.05$), or between AAT activity and total esters ($r = 0.51$, $P > 0.05$) in pawpaw. Although the highest total volatile production was observed after 2 or 4 weeks of cold storage followed by 72 h of ripening (**Table 2**), AAT activities at these times were lower than that shortly after harvest. Thus, substrate availability may be a more

Table 2. Individual Headspace Volatile Profile Compositions of Pawpaw Fruits from the Cultivars 'Taytwo' and 'Wells'[a]

		storage duration (weeks)					contrasts (P)	
volatile compd	ID[b]	0	2	4	6	8	linear	quadratic
ethyl alcohol	RT, GC-MS	2.0	2.0	10.3	9.2	19.8	<0.0001	0.1637
ethyl acetate	RT, GC-MS	0.1	0.1	0.7	2.2	10.0	0.0001	0.0002
ethyl propionate	RT, GC-MS	0.1	1.1	5.2	8.8	20.0	<0.0001	0.0233
methyl butanoate	RT, GC-MS	0.0	0.7	1.7	0.2	0.1	0.7621	0.0005
ethyl butanoate	RT, GC-MS	1.4	67.0	82.9	1.4	1.2	0.4615	0.0254
methyl hexanoate	RT, GC-MS	32.0	47.3	16.6	8.4	2.9	0.0484	<0.0001
hexanoic acid	RT	0.4	0.9	0.8	3.2	17.9	<0.0001	<0.0001
ethyl hexanoate	RT, GC-MS	70.1	316.9	354.1	32.4	10.8	0.1877	<0.0001
methyl octanoate	RT, GC-MS	87.6	92.3	26.6	31.9	11.0	0.2542	0.0124
octanoic acid	RT	2.0	2.8	2.4	8.4	23.6	<0.0001	0.0161
ethyl octanoate	RT, GC-MS	17.2	100.5	159.4	21.3	29.1	0.8125	0.0009
ethyl decanoate	RT, GC-MS	0.5	4.4	5.1	1.1	1.2	0.7631	0.0107
total	CT	232.1	666.5	705.4	158.6	175.3	0.6119	<0.0001

[a] Fruits were ripened for 72 h after harvest (0 weeks) and after removal from cold storage. Data are the mean of both cultivars with $n = 3$ measurements per cultivar on each date. Data are expressed as ng/g of FW. Statistical analysis of the trends in volatile production across storage time by single degree of freedom contrasts is shown. [b] Identification (ID) based on match with retention time (RT) and/or GC-MS spectra compared to authentic standard, or cumulative total (CT) of identified and unidentified volatile compounds with a minimum of 0.002 ng/g of FW.

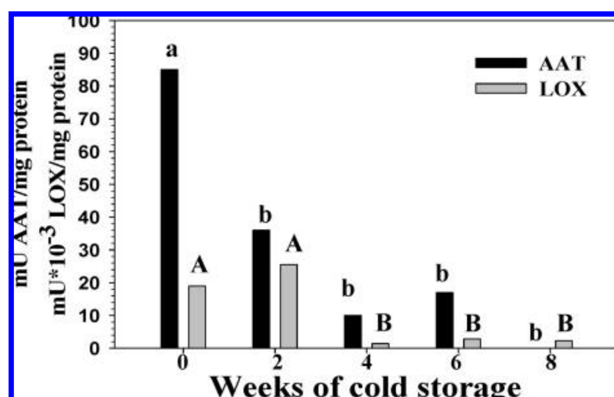

Figure 2. Alcohol acyltransferase (AAT) and lipoxygenase (LOX) activity of 'Taytwo' pawpaw fruit after harvest or after 2, 4, 6, or 8 weeks of cold storage, followed by 72 h of ripening at room temperature. Enzyme activities are expressed as milliunits (mU) per milligram of protein. One AAT or LOX activity unit is defined as the increase in 1 unit of absorbance per minute. Data are expressed as the mean of $n = 3$ measurements. Mean separations among AAT values (lower case letters) and LOX values (upper case letters) by Fisher's LSD at $P = 0.05$ are given.

important factor than AAT activity (*19, 33, 34*).

ADH Activity during Ripening and Cold Storage. Mean ADH activity across storage dates and 4 and 72 h of ripening was 1018 ± 662 milliunits/mg of protein. However, it did not change during ripening after harvest or during cold storage (data not shown).

LOX Activity during Ripening and Cold Storage. LOX activity increased 5-fold during ripening just after harvest or 2 weeks of cold storage, but this ripening-related increase was lost by 4 weeks (data not shown). LOX activity after harvest was generally comparable to that reported for apple (*19*) and strawberry (*35*). LOX activity was low after 4 or more weeks of cold storage (**Figure 2**). There were no significant correlations ($P > 0.05$) of ADH ($r = 0.24$) or LOX ($r = 0.07$) activity with AAT activity or between ADH activity ($r = 0.52$) or LOX ($r = 0.40$) and total volatile production.

Pawpaw fruits ripened normally at ambient temperature after up to 4 weeks of cold storage, but ripening was not normal or failed to occur after longer cold-storage periods. Once moved back to room temperature, fruits stored for >4 weeks exhibited declining levels of respiration and ethylene production, had lower pH and higher soluble sugar content, and produced fewer volatile esters but more off-flavor compounds such as ethyl alcohol, ethyl acetate, and ethyl propionate than fruits cold-stored for ≤4 weeks. These responses suggest that pawpaw fruit are susceptible to chilling injury (*7, 8*). There was no clear relationship between AAT activity and volatile production of pawpaw fruit, suggesting that factors such as substrate availability could play an important role defining pawpaw aroma. Neither ripening for 4 or 72 h nor cold storage had an effect on ADH activity, although LOX activity rose during ripening after harvest and 2 weeks of cold storage. However, neither showed a clear relationship to AAT activity or volatile production. Total aroma production and qualitative similarity to at-harvest profiles, along with fruit firmness and sugar content, confirmed the market quality of pawpaw fruit as a fresh product through 4 weeks of cold storage. Because ripening was not normal after storage at −2 °C and for periods longer than 4 weeks at 2−4 °C, pawpaw fruit should be cold-stored at 2−4 °C for no longer than 4 weeks to retain acceptable quality, although poststorage shelf life will be no more than 3−5 days.

ACKNOWLEDGMENT

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LITERATURE CITED

(1) Archbold, D. D.; Pomper, K. W. Ripening pawpaw fruit exhibit respiratory and ethylene climacterics. *Postharvest Biol. Technol.* **2003**, *30*, 99–103.
(2) Archbold, D. D.; Koslanund, R.; Pomper, K. W. Ripening and postharvest storage of pawpaw. *HortTechnology* **2003**, *13*, 439–441.
(3) Koslanund, R.; Archbold, D. D.; Pomper, K. W. Pawpaw [*Asimina triloba* (L.) Dunal] fruit ripening. I. Ethylene biosynthesis and production. *J. Am. Soc. Hortic. Sci.* **2005**, *130*, 638–642.
(4) Merodio, C.; De La Plaza, J. L. Cherimoya In *Postharvest Physiology and Storage of Tropical and Subtropical Fruits*; Mitra, S., Ed.; CAB International: New York, 1997; pp 269−293.
(5) Koslanund, R. Ethylene production, fruit softening, and their manipulation during pawpaw ripening. Ph.D. Dissertaion, University of Kentucky, 2003.
(6) Alique, R.; Zamorano, J. P.; Calvo, M. L.; Merodio, C.; De la Plaza, J. L. Tolerance of cherimoya (*Annona cherimola* Mill.) to cold storage. *J. Am. Soc. Hortic. Sci.* **1994**, *119*, 524–528.

(7) Saltveit, M. E., Jr.; Morris, L. L. Overview of chilling injury of horticultural crops In *Chilling Injury of Horticultural Crops*; Wang, C. Y., Ed.; CRC Press: Boca Raton, FL, 1990; pp 3−16.
(8) Wang, C. Y., Ed. *Chilling Injury of Horticultural Crops*; CRC Press: Boca Raton, FL, 1990; pp 328.
(9) Duffrin, M. W.; Pomper, K. W. Development of flavor descriptors for pawpaw fruit puree: a step toward the establishment of a native tree fruit industry. *Fam. Cons. Sci. Res. J.* **2006**, *35*, 118–130.
(10) McGrath, M. J.; Karahadian, C. Evaluation of headspace volatiles and sensory characteristics of ripe pawpaw (*Asimina triloba*) from selected cultivars. *Food Chem.* **1994**, *51*, 255–262.
(11) Shiota, H. Volatile components of pawpaw fruit (*Asimina triloba* Dunal.). *J. Agric. Food Chem.* **1991**, *39*, 1631–1635.
(12) Holland, N.; Menezes, H. C.; Lafuente, M. T. Carbohydrates as related to the heat-induced chilling tolerance and respiratory rate of 'Fortune' mandarin fruit harvested at different maturity stages. *Postharvest Biol. Technol.* **2002**, *25*, 181–191.
(13) Polenta, G.; Lucangeli, C.; Budde, C.; Gonzalez, C. B.; Murria, R. Heat and anaerobic treatments affected physiological and biochemical parameters in tomato fruits. *Food Sci. Technol.* **2006**, *39*, 27–34.
(14) Maldonado, R.; Sanchez-Ballesta, M. T.; Alique, R.; Escribano, M. I.; Merodio, C. Malate metabolism and adaptation to chilling temperature storage by pretreatment with high CO_2 levels in *Annona cherimola* fruit. *J. Am. Soc. Hortic. Sci.* **2004**, *52*, 4758–4763.
(15) Hamilton-Kemp, T. R.; Archbold, D. D.; Yu, K.; Collins, R. W. Emission patterns of wound volatile compounds following injury of ripe strawberry fruit. *J. Sci. Food Agric.* **2003**, *83*, 283–288.
(16) Ke, D.; Zhou, L.; Kader, A. A. Mode of oxygen and carbon dioxide action on strawberry ester biosynthesis. *J. Am. Soc. Hortic. Sci.* **1994**, *119*, 971–975.
(17) Defilippi, B. G.; Kader, A. A.; Dandekar, A. M. Apple aroma: alcohol acyltransferase, a rate limiting step for ester biosynthesis, is regulated by ethylene. *Plant Sci.* **2005**, *168*, 1199–1210.
(18) Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principles of protein−dye binding. *Ann. Biochem.* **1994**, *72*, 248–254.
(19) Defilippi, B. G.; Kader, A. A.; Dandekar, A. M. Relationship of ethylene biosynthesis to volatile production, related enzymes, and precursor availability in apple peel and flesh tissue. *J. Agric. Food Chem.* **2005**, *53*, 3133–3141.
(20) Myung, K.; Hamilton-Kemp, T. R.; Archbold, D. D. Biosynthesis of *trans*-2-hexenal in response to wounding in strawberry fruit. *J. Agric. Food Chem.* **2007**, *54*, 1442–1448.
(21) Lyons, J. M.; Breidenbach, R. W. Relation of chilling stress to respiration In *Chilling Injury of Horticultural Crops*; Wang, C. Y., Ed.; CRC Press: Boca Raton, FL, 1990; pp 223−234.
(22) Peterson, R. N.; Cherry, J. P.; Simmons, J. G. Composition of pawpaw (*Asimina triloba*) fruit. *Northern Nut Growers Assoc. Annu. Rep.* **1982**, *73*, 97–107.
(23) McGrath, M. J.; Karahadian, C. Evaluation of physical, chemical and sensory properties of pawpaw fruit (*Asimina triloba*) as indicators of ripeness. *J. Agric. Food Chem.* **1994**, *42*, 968–974.
(24) Berger, H.; Galleti, L. Color as a harvest index for cherimoya. *Acta Hortic.* **2005**, *682*, 1471–1474.
(25) Defilippi, B. G.; Kader, A. A.; Dandekar, A. M. Impact of suppression of ethylene action or biosynthesis on flavor metabolites in apple (*Malus domestica* Borkh.) fruits. *J. Agric. Food Chem.* **2004**, *52*, 5694–5701.
(26) Munoz, T.; Ruiz-Cabello, J.; Molina-Garcia, A. D.; Escribano, M. I.; Merodio, C. Chilling temperature storage changes the inorganic phosphate pool distribution in cherimoya (*Annona cherimola*) fruit. *J. Am. Soc. Hortic. Sci.* **2001**, *126*, 122–127.
(27) Wood, R.; Peterson, S. Lipids of the pawpaw fruit: *Asimina triloba*. *Lipids* **1999**, *34*, 1099–1106.
(28) Taylor, J. A. Exotics In *Biochemistry of Fruit Ripening*; Seymour, G. B., Ed.; Chapman and Hall: London, U.K., 1993; pp 151−187.
(29) Engel, K. H.; Heidlas, J.; Tressl, R. The flavour of tropical fruits (banana, melon, pineapple) In *Food Flavors*; McLeod, M., Ed.; Elsevier Science: St. Louis, MO, 1990; pp 195−201.
(30) McLeod, A. J.; MacLeod, G.; Snyder, C. H. Volatile aroma constituents of mango (cv. Kensington). *Phytochemistry* **1988**, *27*, 2189–2193.
(31) Merodio, C.; Munoz, M. T.; Del Cura, B.; Buitrago, D.; Escribano, M. I. Effect of high CO_2 level on the titres of γ-aminobutyric acid, total polyamines and some pathogenesis-related proteins in cherimoya fruit stored at low temperature. *J. Exp. Bot.* **1998**, *49*, 1339–1347.
(32) Feygenberg, O.; Hershkovitz, V.; Ben-Arie, R.; Nikitenko, T.; Jacob, S.; Pesis, E. Postharvest use of organic coating for maintaining bio-organic avocado and mango quality. *Acta Hortic.* **2005**, *682*, 507–512.
(33) Perez, A. G.; Sanz, C.; Olias, R.; Rios, J. J.; Olias, J. M. Evolution of strawberry alcohol acetyltransferase activity during fruit development and storage. *J. Agric. Food Chem.* **1996**, *44*, 3286–3290.
(34) Wyllie, S. G.; Fellman, J. K. Formation of volatile branched chain esters in banana (*Musa sapientum* L.). *J. Agric. Food Chem.* **2000**, *48*, 3493–3496.
(35) Perez, A. G.; Sanz, C.; Olias, R.; Rios, J. J.; Olias, J. M. Lipoxygenase and hydroperoxide lyase activities in ripening strawberry fruits. *J. Agric. Food Chem.* **1999**, *47*, 249–253.

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