Identification and Quantitation of the Volatile Compounds Responsible for the Aroma of Pawpaw (*Asimina triloba*)

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Abstract

The aim of this study was to perform an exhaustive analysis of the aroma active compounds in pawpaw fruit using gas chromatography-olfactometry (GC-O) on capillary GC columns with a variety of extraction techniques such as solid phase microextraction (SPME) and solvent extraction. The volatile extracts were obtained using headspace solid phase micro-extraction (HS-SPME) for 30 min at two temperatures (23 ℃ and 50 ℃), and by solvent extraction with dichloromethane. The extracts were analyzed using gas chromatography-mass spectrometry (GC-MS) and gas chromatography-olfactometry (GC-O). The SPME extraction at 50 ℃ caused an increase in the levels of Strecker aldehydes (3-methyl butanal, methional, and phenylacetaldehyde). To eliminate potential artifacts, the study focused on SPME at 23 ℃ and solvent extraction as the methods for characterization of the odor-active compounds in pawpaw. Forty-four odor active compounds were detected in the pawpaw fruit, including fifteen compounds that were identified in pawpaw for the first time. Some of the newly reported compounds, with high flavor dilution values include homofuraneol, eugenol, vanillin, acetaldehyde, diacetyl, gamma-octalactone and delta-octalactone. These high intensity odor active compounds, in combination with the many esters, contribute to give the sweet, creamy, mango, pineapple and banana-like character used to describe the flavor of pawpaw fruit. In addition, quantitation of these compounds was achieved in this study. These results provide new understanding into the volatile compounds responsible for the aroma of pawpaw fruit.

Keywords Gas Chromatography – Olfactometry (GC-O), Solid Phase Micro Extraction (SPME), Gas Chromatography – Mass Spectrometry (GC-MS), Aroma Extract Dilution Analysis (AEDA), Aroma, Flavor, pawpaw

Abbreviation (GC-O) Gas Chromatography – Olfactometry (GC-O), Solid Phase Micro Extraction (SPME), Gas Chromatography – Mass Spectrometry (GC-MS), Aroma Extract Dilution Analysis (AEDA), (ppm) parts per million; (ppb) parts per billion; (GC-FID) Gas Chromatography – Flame Ionization Detector

1. Introduction

The perception of aroma is derived from the interaction of volatile compounds with our olfactory receptors at a level above their odor detection threshold (Grosch, 2001). Studies have shown that not all volatile compounds contribute to the aroma. For example, there have been more than 500 volatile compounds identified in coffee; however, the aroma of coffee has been replicated with only 27 compounds (Mayer et al., 2000). The invention of an olfactory port connected to a gas chromatograph has enabled one to identify which volatile compounds are most likely responsible for the aroma of the sample (Fuller et al., 1964). Charm analysis, aroma extract dilution analysis (AEDA) and odor activity value (OAV), were techniques developed to process the GC-O data into relevant odor responses for the aroma of food products (Acree et al., 1984; Grosch, 1993). Soon afterwards, validation of this technique was shown by the process of reconstitution and omission experiments using these identified odor active compounds in the food product (Grosch, 2001). Therefore, the tools and approaches exist to identify the odor important compounds in food products; however, this approach has not been applied to the pawpaw fruit.

The pawpaw fruit is native to the Eastern part of the United States and grows wild in the forest understory

(Zimmerman, 1941). It has broad leaves like tropical plants and produces fruits which have the resemblance of other tropical fruits such as mango, pineapple, and banana (Brannan et al., 2012). The fruit has a green exterior skin and a yellowish-orange flesh with large seeds and resembles a mango; however, the flesh is slightly softer (Figure 1). Upon ripening, the aroma is quite intense and attractive. One attribute which has prevented the commercial distribution of this fruit is its rapid deterioration upon reaching its maturity, typically less than two weeks (Peterson, 1991). Therefore, the most common environment to experience this fruit is at a local farmers market, pawpaw festival, or a restaurant which may prepare special deserts around the pawpaw fruit. One of the popular desert dishes for this fruit is ice cream.

Figure 1. Picture of pawpaw fruit growing as a cluster and a cross-section of the fruit (KSU-Chappell)

Photo credit: Jonathan Palmer, Kentucky State University

Previous work on the volatiles of pawpaw fruit were performed by McGrath and Karahadian (1994a, 1994b). In their research, they captured the pawpaw volatiles on Tenax GC^R traps and eluted with diethyl ether prior to gas chromatographic analysis. They identified several ester volatiles, with ethyl hexanoate, ethyl octanoate, and ethyl butyrate being the predominant compounds based on peak area. In addition, they identified acetoin and gamma-hexalactone in their extracts. They experimented with gas-chromatography-olfactometry (GC-O) on a packed 3 m x 2 mm i.d. silane-deactivated glass column containing 10% SE-54 and were able to detect approximately 14 odorants which included various esters, acetoin, and gamma-hexalactone. Due to the low resolution with a packed column, they hypothesized that additional odor-relevant compounds could have been missed in this early research.

Therefore, the aim of this study was to perform an exhaustive analysis of the aroma active compounds in pawpaw fruit using GC-O on capillary GC columns with a variety of flavor extraction techniques such as solid phase micro extraction (SPME) and solvent extraction. These approaches should provide additional insights into the odor active compounds of pawpaw and provide a better understanding for the tropical character of this odiferous fruit.

2. Materials and Methods

2.1 Samples and Chemicals

Fresh KSU-Atwood™ pawpaw cultivar, picked at ripe stage during late August and early September, was provided by Sheri B. Crabtree from the Kentucky State University (KSU) pawpaw research program (Frankfort, KY). Each pawpaw fruit weighed between 200g and 450g. These samples were picked at the ripe stage from the 2020 harvest. Frozen puree of the 2019 Susquehanna pawpaw fruit was also provided by the KSU pawpaw research program and used for initial GC-O experiments and method development.

Dichloromethane and sodium hydroxide were purchased from Fisher Scientific (Hanover Park, IL, USA) and ethanol was from Greenfield Global (Shelbyville, KY). Acetaldehyde, 2,3-butanedione, 3-hydroxy-2-butanone (acetoin), gamma-octalactone, delta-octalactone, delta-nonalactone, gamma-hexalactone, 5-ethyl-4-hydroxy-2-methyl-3(2H)-furanone (homofuraneol), ethyl butyrate, ethyl hexanoate, ethyl heptanoate, ethyl octanoate, methional, acetic acid, butyric acid, hexanoic acid, octanoic acid, decanoic acid, phenylacetic acid, 2-isobutyl-3-methoxypyrazine, methyl cinnamate, eugenol, coumarin, vanillin, geraniol, citronellol, methyl octanoate, ammonium sulfate, and alkane standard (C7-C30) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ultra-pure water was sourced from a Milli-Q system (Millipore, Bedford, MA, USA). Acetic acid-d$_4$, acetaldehyde-d$_4$, 2,3-butanedion-d$_6$, decanoic acid-d3, octanoic acid-d$_{15}$, hexanoic acid –d$_{11}$, and butyric acid-d$_7$ were purchased from CDN Isotopes (Quebec, Canada).

2.2 SPME Fiber and Extraction Conditions for Pawpaw

The 3-phase SPME fiber, 2cm DVB/CAR/PDMS (Supelco, Bellefonte, PA, USA) was chosen for the headspace extraction of pawpaw volatiles as this fiber has been shown to extract the widest polarity of volatiles and for its proven capability of extracting flavor molecules from fruit samples (Al-Taher & Nemzer, 2020; Zhang et al., 2021). A sample from each individual fresh pawpaw fruit was weighed separately and the frozen pawpaw puree was also weighed separately for analysis. For SPME analysis 2.5 g sample was placed in a clear 20ml screw-cap vial with PTFE septa (Pal Parts, Raleigh, NC). The extractions were performed using a Gerstel MPS SPME autosampler (Gerstel, Linthicum, MD, USA). An equilibration time of 10 min was followed by 20 min at either 23 ℃ and 50 ℃, using the heating agitator. Then, the fiber was desorbed into an Agilent split/splitless GC inlet operated 250 ℃ in splitless mode for 6 min. Each GC-O panelist performed 3-5 runs on each fruit and the puree at both temperatures.

2.3 Sample Preparation for Aroma Extract Dilution Analysis (AEDA)

To prepare an extract, three ripe pawpaw fruits were combined, 100 g of pawpaw fruit and 200 g of deionized water was homogenized in a blender (Waring ¾ HP commercial blender, McConnellsburg, PA, USA). The solution was then centrifuged in an Eppendorf 5804R centrifuge at 3000 rpm for 20 min to remove solids. The clear solution was transferred to 50 mL glass conical centrifuge vials. 10 g of ammonium sulfate and 6 g of dichloromethane were added to 30 g of pawpaw supernatant. The sample was inversion mixed by hand for 10 min followed by 1 min on the vortex mixer. The sample was centrifuged at 1500 rpm for 10 min; the dichloromethane layer was removed with a glass Pasteur pipet and transferred to a separate glass vial. A second and third extraction of the pawpaw supernatant was performed identical to the first and the dichloromethane extracts were combined (recovered 14 g). Gas Chromatography-Olfactometry (GC-O) was performed on this initial extract prior to concentration to identify potential volatile compounds that could be loss during the concentration step. Acetaldehyde, diacetyl, and homofuraneol were detected in the initial extract prior to concentration. The extract was concentrated to ~100 μL using a Biotage TurboVap LV and 1 μL was injected splitless into the GC for olfactory analysis. Aroma Extract Dilution Analysis (AEDA) was performed on the sample using the Agilent GC-MS coupled to a Gerstel Olfactory Detection Port, ODP3. GC-O was performed on this concentrated extract and then the volume was increased two-fold, successively: 0.125, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 16, 32, 64 and 128 mL which corresponded to 1, 2, 4, 8, 16, 32, 64, 128, 256, 512, and 1024x dilution (Grosch 2001). Odor active compounds were given a flavor dilution factor (FD) based on the last dilution step that the odor was detected by GC-O. For example, if an odor compound was detected at the 4-dilution step (2.0 mL) then it would have a FD factor of 16.

2.4 Quantitation

Quantitation of the aroma compounds in pawpaw was achieved using two approaches: Stable Isotope Dilution Analysis (SIDA, Schieberle, 1995) and Gas Chromatography-Flame Ionization Detection (GC-FID) using relative response factors according to Cachet et. al (2015). SIDA was used for the analysis of acetaldehyde, diacetyl, acetic acid, butyric acid, hexanoic acid, octanoic acid, and decanoic acid. GC-FID was used for quantitation of all other compounds. The Agilent 7820a gas chromatograph column was split 2:1 (FID: MS) between an FID and Agilent 5977 mass spectrometer (MS) using the Gerstel uFlow Manager®. This setup enabled us to determine that compounds were well separated on the column and had no interference as determined by evaluation of the mass spectral data. Quantitation was achieved using calibration curves generated as a plot of ratio of concentration of analyte to the concentration of internal standard (isotope for SIDA or methyl octanoate and ethyl heptanoate for GC-FID) vs. ratio of peak area response of analyte to internal standard. All calibration curves achieved correlation coefficients of $R^2 > 0.99$, except for acetaldehyde and diacetyl which exhibited $R^2 > 0.97$ and $R^2 > 0.95$ respectively. These techniques were used to quantitate the concentration of volatile compounds in the pawpaw fruit. Quantitation analysis was performed in triplicate (aliquots of different samples) and data is reported with standard deviations. The pawpaw sample preparation was identical to the AEDA approach above, and internal standards were added to the pawpaw sample prior to homogenization with blender. This would account for sample compound loss during centrifugation steps to remove solids.

For trace compound analysis, the pawpaw extract pH was adjusted to pH 8.0-9.0 with 0.1 N sodium hydroxide prior to extraction with dichloromethane. This step removed the acids and provided a cleaner chromatogram for quantitation of trace compounds.

2.5 Gas Chromatography-Mass Spectrometry (GC-MS)

The analysis of aroma volatiles extracted by HS-SPME, and liquid injection was performed using a Model 7820A gas chromatograph (GC) equipped with a 5977 mass spectrometer detector (MSD) and Flame Ionization

Detector (FID) from Agilent (Agilent Technologies, Santa Clara, CA, USA). Olfactometry was performed using the Gerstel Olfactory Detection Port (ODP 3) which was connected to the Agilent 7820a gas chromatograph (GC) with the Agilent 5977 mass spectrometer (MS). The spilt ratio was 2:1 (olfactory port : MS) using the Gerstel UFlowManager®. The GC was coupled with a Gerstel Multipurpose Sampler (MPS) with SPME capability (Linthicum, MD, USA). The injector port had a 0.754 mm deactivated GC liner, and the inlet was kept at a constant temperature of 250 ℃. A fused silica HP-5ms-UI column (30 m x 0.25 mm ID x 0.25 μm thick film) and J&W DB-Wax (30 m x 0.25 mm ID x 0.25 um film thickness) Agilent Technologies (Santa Clara, CA, USA) were used for analysis. Helium was the carrier gas with a constant flow rate of 1 mL/min. The initial oven temperature was 50 ℃ with a hold time of 1 min. Then the temperature rose to 240 ℃ at 15 ℃/min then held for 5 min. The MSD operated in electron ionization mode at 70 ev. The MSD transfer line was set at 280 ℃. The ion source was heated at 230 ℃ and the MS quads were heated at 150 ℃. SPME was performed without solvent delay; however, a 3.5 min solvent delay was used for liquid injections. The mass acquisition range was 35 to 250 m/z.

2.6 Olfactometry

Four olfactory panelists were trained in GC-O and odor recognition; each performed 3 replications. Intensity of odor compounds was rated on a 9-point scale (low, medium, strong; – and +). For example, medium can be medium -, medium, or medium +. An aroma peak was determined to be aroma active if it was detected with at least half of the analyses. For the AEDA analysis, FD factors were based on an average response for last dilution detection of odor from the four panelists.

2.7 Compound Identification

Compound identifications was determined by a combination of retention indexes, mass spectra comparison with libraries (NIST 14, FFNSC3), odor description, and confirmation by injecting authentic standards on the same columns. Alkane linear retention indexes were obtained using a (C_7-C_{30}) alkane standard mixture. Linear retention indexes of aroma compounds were calculated on both a DB-5 and DB-Wax GC columns.

2.8 Data Analysis

Aroma volatile compound identification and quantitation were performed using Agilent Technologies' ChemStation software (version F.01.03). Microsoft Excel 2016 (Redmond, WA, USA) was used for the calculation of means, standard deviations (SD) and relative standard deviations (%RSD).

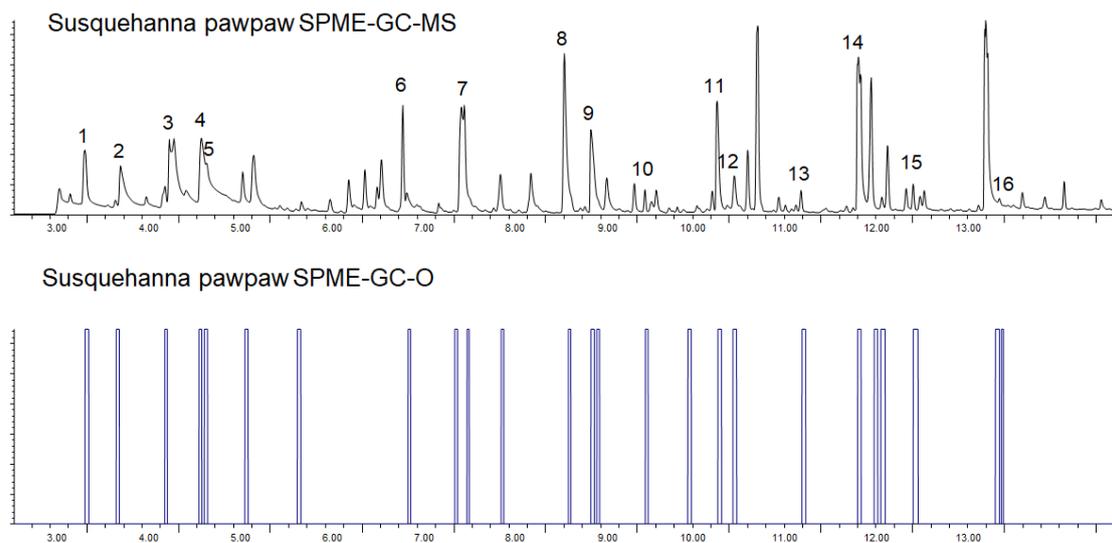## 3. Results and Discussion

3.1 Aroma-active Compounds in Pawpaw

Figure 2. SPME-GC-MS-O of Susquehanna pawpaw fruit at 50 ℃ for 20 min

(1) acetaldehyde, (2) 2-methylpropanal, (3) 3-methylbutanal, (4) diacetyl, (5) ethyl butyrate, (6) ethyl hexanoate, (7) acetoin, (8) ethyl octanoate, (9) acetic acid, (10) linalool (11) butyric acid (12) phenylacetaldehyde, (13) citronellol, (14) hexanoic acid, (15) gamma-octalactone and (16) homofuraneol.

The initial approach for odor analysis of the pawpaw sample was by using SPME-GC-MS-O as described in materials and methods. Figure 2 shows a typical chromatogram and olfactory response for pawpaw analysis by SPME at 50 ℃. In this analysis, many aroma-active compounds were identified such as esters, Strecker aldehydes, lactones, homofuraneol, and straight chain acids. The three-phase SPME fiber (DVB/CAR/PDMS) provided a substrate for the extraction of various aroma compounds and is an excellent choice for capturing both polar and nonpolar compounds, as demonstrated in this analysis. In addition, this technique enabled us to detect extremely volatile compounds because there is no solvent involved and we can perform GC-O immediately following injection. Using this approach, we were able to detect acetaldehyde, 2-methyl propanal, and diacetyl which are typically missed in solvent extraction techniques, as they elute under the solvent peak.

When performing SPME at 50 ℃ on the pawpaw fruit, we noticed that the fruit was slightly darker following extraction. In addition, we were concerned with the significant levels of Strecker aldehydes (2-methyl propanal, 3-methyl butanal, methional, and phenylacetaldehyde) as shown in Figure 2. It is likely that that some of these compounds could be developed during the sample process. Therefore, the SPME extraction was repeated at room temperature, which resulted in these compounds still being detected; however, the levels were much lower. With these learnings, it was decided that further GC-O and quantitation experiments would be done using liquid-liquid extraction. This initial experiment provided evidence for new, unreported odor active compounds in the pawpaw fruit.

3.2 Aroma Strength of the Odor-active Compounds

To get a better understanding of the aroma strength of each odor active compound, the KSU-Atwood™ pawpaw sample was analyzed by AEDA. Table 1 shows the results for this analysis of in order of elution on the GC wax column. We were unable to obtain mass spectra for three compounds (methional, 2-isobutyl-3-methoxypyrazine, and 2-acetyl-1-pyrroline); however, their identification was based on retention index, odor description and comparison with standard. Homofuraneol and diacetyl had the highest dilution values followed by acetaldehyde, gamma-octalactone, ethyl butyrate, ethyl hexanoate, methional, butyric acid, hexanoic acid, eugenol, coumarin, and vanillin. Accurate identification of homofuraneol was facilitated by mass spectra fragmentation patterns provided in publication (Fay et al., 1997).

Of these top twelve odor impactful compounds, eight have not been cited before in relevance to the aroma and flavor of pawpaw fruit. In addition, other interesting compounds with substantial odor importance are 2-acetyl-1-pyrroline, geraniol, citronellol, linalool, delta octalactone, and delta nonalactone. These lactones are newly reported in pawpaw. Citronellol and geraniol had been previously identified in pawpaw varieties in Japan (Shiota 1991). However, the delta lactones are newly discovered in pawpaw and contribute to the creamy, lactonic character of this fruit. The major odor active compounds have been identified, but there are still some trace unidentified odor compounds which exhibited caramelized sugar and lactonic character (Table 1). These compounds exhibited FD levels of 16 or lower and their contributions are complementary and subtle to the overall flavor of highest aroma active compounds identified in pawpaw.

Table 1. Aroma active compounds in KSU-Atwood[TM] pawpaw fruit identified by AEDA

No	Compound ID	Retention Index		Odor Description	FD Factor
		Wax	DB-5		
1	acetaldehyde	<800	<800	sweet, fruity, fresh	512
2	diacetyl (2,,3-butanedione)	1001	<800	buttery	1024
3	ethyl butyrate	1055	807	fruity	512
4	ethyl hexanoate	1246	1001	fruity	256
5	unknown	1336		earthy	8
6	2-acetyl-1-pyrroline	1363	923	roasted, com chip	128
7	ethyloctanoate	1448	1200	ester; tequilla note	16
8	unknown	1457		woody	16
9	unknown	1466		plastic	16
10	acetic acid	1474	<800	solvent like, glue	16
11	methional	1484	908	potato	256
12	3-hydroxy ethyl butyrate	1535		fruity, buttery, cheesy	16
13	2-isobutyl-3-methoxy pyrazine	1552	1195	woody, earthy	16
14	linalool	1554	1102	sweet, floral	16
15	3-hydroxy ethyl hexanoate	1615		watermelon note	16
16	unknown	1636		woody, stinky	16
17	unknown	1644		fruity	8
18	butyric acid	1659	850	cheesy	512
19	gamma hexalactone	1749		carmelized sugar note	8
20	citronellol	1784	1235	floral, sweet	32
21	gerianol	1869	1279	floral	128
22	hexanoic acid	1876	1085	stinky acid	256
23	unknown (lactone m/z=99)	1898		clovish, spice, sweet	64
24	unknown	1916		spicy, eugenol like	16
25	gamma-octalactone	1962	1261	lactone, coconut	512
26	delta-octalactone	2021	1310	lactone, creamy	12S
27	unknown	2077		spicy, licorice-like	32
28	octanoic acid	2087	1275	waxy	32
29	homofuraneol	2100	1158	cotton candy	1024
30	methyl cinnamate	2122		strawberry	16
31	p-cresol	2167	1084	stinky, indole like	8
32	eugenal	2207	1368	spicy note cinnamon	256
33	delta-nonalactone	2212	1397	sweet, creamy	16
34	unknown	2252		creamy, lactone, carmelized	16
35	unlutown	2288		indole like	16
36	decanoic acid	2308	1380	waxy	16
37	unknown	2398		carmelized note	16
38	unknown	2422		carmelized note	lfi
39	unknown	2462		lactone, creamy	16
40	unknown	2519		phenolic, waxy	8
41	coumarin	2557	1444	soapy perfumery	256
42	vanillin	2665	1415	vanillin	256
43	phenylacetic acid	2707		woody, floral	16
44	dihyrocinnamic acid	2754		woody	16

The aroma of pawpaw has been described as like a banana, mango, and pineapple. Some of the major aroma compounds in pawpaw are also present in pineapple. The compounds gamma-octalactone, delta-octalactone, and ethyl hexanoate which were found in our study are also key aroma compounds in pineapple (Zheng, L-Y et al., 2012). In addition, homofuraneol was found in pawpaw and pineapple has furaneol as a key aroma compound. Homofuraneol, which is the ethyl derivative of furaneol, has very similar aroma qualities. In respect to mango, there were more similarities in the aroma compounds. Mangoes have been characterized using GC-O and the following compounds are shared with pawpaw: gamma-octalactone, delta-octalactone, delta-nonalactone,

geraniol, ethyl butyrate, diacetyl, furaneol (homofuraneol in pawpaw), and vanillin (Kuroki, R. et al., 2021). With regards to bananas, pawpaw share eugenol, ethyl hexanoate, and ethyl butyrate (Jha, S.K. et al., 2021; Jordan, M.J. et al., 2001). Upon evaluation of the similarities in aroma compounds between pawpaw and mangoes, pineapples, and bananas, it is reasonable to believe that pawpaw could have similar aroma qualities to all three of these fruits. Therefore, it is reasonable to see how pawpaw fruit could be compared to the flavor characteristics of these other three fruits. In addition, pawpaw fruit shares many of the aroma active compounds found in dairy products (Drake et al., 2010) such as homofuraneol, butyric acid, diacetyl, and acetoin. These similarities could help explain the popularity of pawpaw ice cream as the main desert application for this fruit.

3.3 Major Odor-active Compounds in Pawpaw

Quantitation of the main aroma compounds was carried out using a combination of Stable Isotope Dilution Analysis (SIDA) with isotopically labeled internal standards for seven compounds and by GC-FID using relative response factors for the remaining compounds as outlined in the methods section. Table 2 shows the quantitation for 26 of the top 29 odor active compounds in the KSU-Atwood[TM] pawpaw fruit. As shown, there are large differences between the concentrations of the various odor active compounds. Nonetheless they are all important in contributing to the flavor of pawpaw. Many of these compounds have low odor thresholds, which enables those compounds to contribute strongly even at low concentrations in a food product. Gamma-octalactone, which has a reported taste threshold of 7 ppb (Leffingwell), was determined to be present at 102 ppb. This value is more than ten-times the taste threshold, supporting its importance as a key flavor compound in the KSU-Atwood[TM] pawpaw fruit.

Table 2. Quantitation of major odor active compounds in KSU-Atwood™ pawpaw fruit

Compound	Concentration ppb (ug/kg)	Flavor Dilution
diacetyl	4232 ± 393	1024
homofuraneol	6500 ± 1178	1024
acetaldehyde	211000 ± 25320	512
gamma-octalactone	102 ± 3.2	512
ethyl butyrate	7854 ± 212	512
butyric acid	3120 ± 159	512
ethyl hexanoate	24360 ± 828	256
methional	*ND**	256
hexanoic acid	23900 ± 717	256
eugenol	34 ± 4.5	256
coumarin	5 ± 1.5	256
vanillin	11 ± 1.2	256
2-acetyl-1-pyrroline	*ND**	128
gerianol	27 ± 0.8	128
delta-octalactone	64 ± 2.3	128
citronellol	65 ± 4.0	32
octanoic acid	73800 ± 4904	32
ethyl octanoate	1800 ± 41	16
acetic acid	6500 ± 182	16
3-hydroxy ethyl butyrate	5015 ± 187	16
2-isobutyl-3-methoxypyrazine	*ND**	16
linalool	120 ± 6.1	16
3-hydroxy ethyl hexanoate	8214 ± 344	16
methyl cinnamate	39 ± 1.3	16
delta-nonalactone	7 ± 0.7	16
decanoic acid	3900 ± 117	16
phenyl acetic acid	3 ± 0.24	16
dihydrocinnamic acid	75 ± 4.8	16
gamma hexalactone	328 ± 24	8

*ND: none detected, below instrument detection limit. Limit of detection (0.1 ppb); Limit of quantitation (1ppb).

In this study, we had to overcome a few challenges in the quantitation. As shown in Table 2, we had to perform quantitation on compounds that were present at varying levels. To accomplish that task, GC-FID analysis was

performed on the initial extract to obtain quantitative accuracy for the more abundant compounds (acetaldehyde, ethyl hexanoate, and acids), followed by concentration and reinjection in order to achieve signal for less abundant compounds. The pH adjustment (basic) prior to extraction in order to remove acids was beneficial in quantitation of the trace compounds. Even though some compounds were easily detected by GC-O, their quantitation was challenging. This problem existed for methional, 2-acetyl-1-pyrroline, and 2-siobutyl-3-methoxypyrazine which proved to be below our instrument detection limits. Due to limited samples, we were unable to perform a larger extract which will be required for obtaining a mass spectrum for these compounds. In addition, the stable isotope dilution analysis approach may be required for these trace compounds as well. Overall, quantitation was achieved on 26 of the major aroma active compounds.

4. Conclusion

This study revealed new insights into the important aroma active compounds in pawpaw fruit. Using GC-O on both headspace (SPME) and solvent extraction samples enabled us to detect 15 new aroma active compounds identified for the first time in pawpaw fruit. In addition, the discovery of the high AEDA value aroma compounds homofuraneol, diacetyl, lactones, vanillin and coumarin in pawpaw fruit provided additional understanding to some of its unique aroma and flavor qualities. These compounds together with the ethyl esters identified provided a basis for the fundamental flavor of pawpaw fruit. Quantitation was achieved on 26 of the major odor active compounds. This data provides additional knowledge for next steps such as reconstitution studies for validation and sensory evaluation. There are at least 55 different pawpaw varieties growing in the United States. Sensory analysis has described a few of these varieties as having significantly different flavor profiles (Greenawalt, 2016). Further studies could include evaluation of the most extreme flavor variants by GC-O and quantitation. Another study identified sensory differences among pawpaw cultivars (Brannan et al., 2012) and this may be explained by variations in the levels of the important aroma compounds presented in this work. In summary, this work led to the discovery of key aroma active compounds in pawpaw and provided insights for further flavor research of the different cultivars.

Author's contributions

Li Li Zyzak conceived, carried out the experiments and wrote the manuscript; Joshua Zyzak, Nathaniel Britt and Mackenzie Roark contributed to the GC-O and quantitation data. Sheri Crabtree and KSU pawpaw program kindly provided the pawpaw fruits upon harvest.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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