ASSESSMENT AND CLASSIFICATION OF VOLATILE AND PHYTONUTRIENT PROFILES FROM *CUCUMIS MELO* L. BREEDING LINES FOR IMPROVED QUALITY, FOOD SAFETY AND HEALTH BENEFITS USING ADVANCED ANALYTICAL APPROACHES

A Thesis

by

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ABSTRACT

Cucumis melo L. is one of the most commercial and economical crops in the world with several health beneficial compounds as such carotenoids, amino acids, vitamins A and C, minerals and dietary fiber. In the first study, we aimed to characterize volatile organic compounds (VOCs) in 28 melon breeding lines (BL) providing useful information for improving fruit flavor and aroma. The VOCs were identified and characterized using head space solid-phase microextraction (HS-SPME) coupled with gas chromatography-mass spectrometry (GC-MS) and 113 VOCs, including 15 esters, 27 aldehydes, 35 alcohols, 14 ketones, 4 acids, 10 hydrocarbons, 5 sulfurs, and 3 other compounds were identified. The highest average contents of all the VOCs were found in BL-30 (13973.07 µg/kg FW) and the lowest was in BL-22 (3947.13 µg/kg FW). The highest number of VOCs was present in BL-20 (77) and the lowest in BL-22 (42). BL-9 had high levels of carotenoid-derived VOCs. The compounds with the highest contents were benzaldehyde, geranylacetone, and β-ionone. Quality parameters such as color and sugar contents of melons were also measured. All the melon color readings were within the typically acceptable limits due to the presence of carotenoids and β-carotene. BL-22 and BL-14 had the highest (55.54 mg/g FW) and lowest (19.17 mg/g FW) sugar contents, respectively. Principal component analysis (PCA) produced diverse clusters of breeding lines and different chemical classes that contributed to flavor and aroma. BL-4, BL-7, BL-12, BL-20, and BL-30 were selected as important breeding lines based on their organoleptic, antimicrobial and health-beneficial properties. This work thus provides useful information for breeding to improve melon flavor and aroma properties according to consumer preferences, and to improve the flavor, aroma and antimicrobial properties for food safety.

In the second study, we aimed to identify the VOCs along with phytonutrient profiles such as sugars and pH as well as carotenoid contents and antioxidant activities in the melon BL grown

in 2018 and 2019 at Uvalde, Texas. We identified 72 VOCs from 2018 lines and 109 VOCs from 2019 breeding lines. The radical scavenging activities were determined using the total phenolic contents (TPC), 2,2-azino-bis(3-ethyl-benzothiazoline-6-sulfonic acid (ABTS) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assays, carotenoid and sugars were analyzed using high-performance liquid chromatography (HPLC). PCA was used to correlate the breeding lines with VOCs. Major VOCs identified in the breeding lines from both the years were geranylacetone, benzyl alcohol, D-limonene, β-ionone, benzaldehyde, (E)-2-nonenal and citral. BL-96, BL-136 and BL-53 were the most prominent breeding lines observed from 2018, based on the quality parameters such as pH, sugar, and citric acid levels as well as their antioxidant activities, antimicrobial properties and carotenoid concentrations. From the year 2019, the lowest pH (5.82) was observed in BL 3-43-1 along with the highest total sugar content, making it noteworthy. Along with BL 3-43-1, the lines 3-31-1, 2-44-1, 2-42-1 and 3-22 can be considered for future breeding programs based on their organoleptic profiles, high radical scavenging activities and high carotenoid concentrations.

DEDICATION

I would like to dedicate this thesis to my parents, who have always supported me and encouraged me to keep pushing forward, for always giving me strength and motivating me to pursue my dreams, and to my best friend who has been a constant source of support throughout this entire journey.

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Contributors

This work was supervised by my graduate committee: Dr. Bhimanagouda S. Patil from the Department of Horticultural Science and Department of Food Science & Technology, Dr. Kevin Crosby from Department of Food Science and Dr. Rhonda Miller from Department of Animal Science at Texas A&M University, College Station, Texas, USA. All the work was carried out and accomplished by the student under the supervision of the committee.

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CHAPTER I

INTRODUCTION

Muskmelon or cantaloupe melon (*Cucumis melo* L.) belongs to the family of *Cucurbitaceae* and is supposedly originated in Asia.[1] The United States (US) ranks first in the per capita consumption for melons as fresh fruit. One of the factors responsible for this may be the fact that consumers have started to notice many health benefits associated with melon consumption. For example, the Dietary Reference Intake (DRI) for β-carotene is ranked first in melons, it is within the top three fresh fruits consumed for DRI of folic acid and potassium (K) content and within the top four fresh fruits for DRI of folic acid content.[2] According to the U.S. Food and Drug Administration (FDA), melons could be described as fruits that are low in calories, sodium and fat. In addition, 1-cup or 236 gm of muskmelon is known to provide the recommended dietary allowance (RDA) for vitamins A and C.[3]

The words "phytonutrients" or "phytochemicals" have gained popularity due to the consumption of fruits and vegetables, being just more than nutrition and as natural alternatives to medicine.[3] Phytonutrient means a nutrient that is derived from plants. Therefore, lipids, proteins, carbohydrates as well as essential minerals and vitamins are considered as phytonutrients. The organic and inorganic compounds in plant foods that affect human health and nutrition are also considered to be phytonutrients. These would more commonly include nutrients and vitamins such as vitamin E, carotenoids, iron, zinc and calcium.[4] Studies suggest that phytonutrients could be major determinants in creating designer foods contributing to the dietary prevention of chronic diseases.[5] Some of the major roles played by phytonutrients in human health include acting as antioxidants, antibacterial, antifungal, anti-inflammatory and chemo-preventative agents.

Primary and secondary plant metabolites are responsible for various biological and functional properties. Volatile organic compounds (VOCs) are generated by both primary and secondary plant metabolites. So far, more than 7000 VOCs have been identified from foods and beverages. A single fruit or vegetable can synthesize hundreds of volatiles, however, not all of them are recognizable by humans and animals. Only a small percentage of these generate the flavor fingerprint which helps animals and humans recognize and make good food choices while avoiding dangerous ones. The positive perception of foods is said to be correlated by both health and volatiles.[7]

A variety of human foodborne pathogens such as *Listeria monocytogenes*, *Salmonella* spp., *Staphylococcus aureus* and *Escherichia coli* are most commonly associated with fresh fruits and minimally processed refrigerated foods.[8] The shelf-life and safety of foods can be improved considerably by using plants and plant products. Previous studies have demonstrated the use of VOCs isolated from plants acts as a defense system to eliminate the aforementioned pathogens in fresh produce.[10] Most of the reported VOCs are generally recognized as safe (GRAS). Some of these compounds include D-limonene, caprylic acid, benzaldehyde, benzyl alcohol and β-ionone. Therefore, aroma compounds could not only be useful in assessing the flavor quality but also in increasing the shelf-life and safety of fresh produce and minimally processed foods.[9-11]

OBJECTIVES

1. To characterize VOCs and elucidate their relationship to flavor, aroma and antimicrobial properties from *Cucumis melo* L. breeding lines, grown in Weslaco (Texas, USA).

2. To assess and characterize volatile profiles, pH, citric acid activity, sugars, carotenoid concentrations and antioxidant activities from *Cucumis melo* L. breeding lines grown in Uvalde (Texas, USA).

CHAPTER II

LITERATURE REVIEW

Cucumis melo L. is more commonly referred to as cantaloupe or muskmelon belongs to the Reticulatus group and is a member of the family *Cucurbitaceae*.[1] It is one of the most economically important crops grown in the world. While most of the consumer preferences for this fruit are determined based on its sweetness, other important parameters include aroma, texture and the phytonutrient content of the fruit. Previous studies have demonstrated the accumulation of carbohydrates and bioactive compounds in the ripe muskmelon fruits which could be useful for increasing its nutritional value making it a rich source of dietary antioxidants.[12]

Volatile organic compounds (VOCs) in fruits are important determinants of the quality of fruit. Cantaloupe melons are found to have a high concentration of sulfur containing esters and are usually associated with sweet, fruity and floral aromas. Honeydew melons are also high in sugar content while Galia melons are found to exhibit cucumber-like flavor and aroma. Volatile compounds such as (Z)-6-nonenal, ethyl benzoate, ethyl hexanoate, benzaldehyde, benzyl alcohol, are known to be the major constituents of cantaloupe aroma.[13, 14]

VOCs have also been gaining popularity as functional foods, acting as natural antimicrobial alternatives to chemical treatments.[10] In addition to their flavoring properties, VOCs have shown promising results in improving human health and exhibiting anti-inflammatory, anti-cancer and antioxidant activities. Previous reports have found that terpenoids, phenolics and alkaloids are the most potential sources that could act as antimicrobial agents and health-promoting compounds.[15] Compounds such as hexanal, (*E*)-2-hexenal, benzaldehyde, D-limonene, eucalyptol and α-terpineol are found to be effective against microorganisms such as *L. monocytogenes*, *S. enterica*, *E. coli* and *S. aureus*.[16, 17]

Previous studies suggest that fruits and vegetables have several compounds with antioxidant activities, such as carotenoids, vitamins C and E, chlorophylls and phenolic compounds. Based on their composition, antioxidants can be classified as lipophilic and hydrophilic.[18] Free radical scavenging activity can affect if phytonutrients can function in an antagonistic or synergistic way thereby affecting its nutritional and health beneficial properties.[20] Antioxidants can help prevent various diseases such as cardiovascular diseases, inflammation and diabetes by producing reactive oxygen species (ROS).[19, 20] The effects of nutritional antioxidants and the implications of ROS in cardiovascular diseases have been studied extensively.[129] Levels of antioxidants such as carotene and vitamin C in the blood cells are known to increase with the consumption of fruits and vegetables, thereby decreasing cholesterol.[130, 131] The free radical scavenging activity is usually determined using DPPH and ABTS assays, they are known to work on hydrogen atom transfer and single electron transfer respectively.[21]

Antioxidant activities can also be linked to the structure of amino acids. Chelation of metal ions, radical scavenging activity and breakdown of hydroperoxide into free radicals are some of the antioxidant activities performed by amino acids.[22] A signaling molecule such as γ-aminobutyric acid (GABA) can act against ROS.[23] Previous studies reported the role of carotenoids in fruits and vegetables and how it affects human health. For example, β-carotene was found to be the most prevalent of all carotenoids in most fruits and vegetables included in the study. β-cryptoxanthin was associated with various fruits like cantaloupes, oranges, peaches, apricots, grapefruits and nectarines.[24]

Based on the above literature, it is important to characterize compounds from melons that could not only provide us with flavor and aroma attributes but also focus on antimicrobial and antioxidant activities along with health-beneficial compounds. Production of such high-quality

melons will greatly align with consumer demands and preferences, increasing sustainability, profitability and industry-driven needs.

CHAPTER III

ASSESSMENT AND CLASSIFICATION OF VOLATILE PROFILES IN MELON BREEDING LINES USING HEADSPACE SOLID-PHASE MICROEXTRACTION COUPLED WITH GAS CHROMATOGRAPHY-MASS SPECTROMETRY

INTRODUCTION

The average American consumes ≈13 kg of melon each year. The most important melon traits associated with consumer preference are flavor, color, aroma, texture, juiciness, phytonutrients, and sweetness.[27, 28, 29]

The sweetness and phytonutrient contents of melons are important quality attributes. Glucose, sucrose, and fructose make up the total sugar content (~97%) of melons. Sucrose accumulates as the major sugar (~50%) during ripening, with lower levels of fructose and glucose.[28] Muskmelons also have a large number of bioactive compounds such as folic acid, vitamin A (β-carotene), vitamin C (ascorbic acid), L-citrulline (a non-essential amino acid), and micronutrient elements such as iron, magnesium, and potassium.[20, 26] These phytonutrients have numerous health-promoting properties such as anti-inflammatory, analgesic, antioxidant, anticancer, diuretic, antimicrobial, and antidiabetic activities.[29, 30, 31] Therefore, melon breeders have focused on producing improved melon cultivars with high sugar levels and enhanced phytonutrient contents.

In addition to sugar and phytonutrients, horticultural breeding programs have focused on long shelf life and improved food safety, especially for melons.[32] Indeed, the 2011 cantaloupe-related foodborne illness outbreak caused by *Listeria monocytogenes* was the deadliest outbreak in recent US history and caused a 32% reduction in the production of melon in the past decade. In

addition, 43 outbreaks were associated with cantaloupe between 1998 and 2018.[33-36] Plant volatile organic compounds (VOCs) have antimicrobial properties and help protect fruits against decay microorganisms.[37] Phytochemicals, including a wide range of VOCs, contribute to flavor and have been used as natural alternatives to improve the shelf life and safety of food.[10] Melon breeding programs aim to enhance the sustainability and profitability of melon production in the US by concentrating on consumer preferences and industry-driven needs. Therefore, exploration of melon VOC profiles provides key information for breeding programs aimed at producing safe, high-quality melons.

Continuing research on melon VOCs has already identified around 291 volatile compounds.[38] The key contributors to the total VOCs are esters, aldehydes, and ketones, along with smaller quantities of alcohols, sesquiterpenes, and sulfur-containing compounds.[39, 40, 41] These research programs have used various analytical techniques to identify and measure VOCs, including dynamic headspace extraction,[42] stir bar sorptive technique[43] and solid phase microextraction (SPME).[44, 45] Head space (HS)-SPME is a simple, solvent-free method for extraction and concentration of volatile compounds, combined with gas chromatography-mass spectrometry (GC-MS). We used this technique to analyze the VOCs in various citrus fruits.[46, 47]

Although several studies have evaluated the VOCs from melon cultivars,[14, 44, 48] our knowledge of the VOC profiles of breeding lines in melon remains limited. Therefore, this study aimed to (a) characterize the VOCs from 28 melon breeding lines, (b) improve our knowledge about the aroma profile and VOC composition of various breeding lines, and (c) elucidate the relationship between volatile compounds and flavor, antimicrobial properties, and sugars.

MATERIALS AND METHODS

Plant materials

In this study, 28 melon breeding lines were cultivated in the year 2019 at the Texas AgriLife Research and Extension Center, Weslaco, Texas between March and July. All the fruits were transported to Vegetable and Fruit Improvement Center (VFIC), College Station, Texas, for further analysis.

Chemicals and reagents

Gas chromatographic and HPLC results were verified using authentic standards including (*R*)-(+)-limonene, 2-pentylfuran, 6-methylhept-5-en-2-one, ethyl heptanoate, 1-hexanol, dimethyl trisulfide, (*Z*)-3-hexan-1-ol, ethyl caprylate, 1-octen-3-ol, decanal, benzaldehyde, ethyl butyrate, (*E*)-2-heptenal, (*E*,*Z*)-2,6-nonadienal, (*E*)-carveol, geranylacetone, β-ionone, benzothiazole, thymol, eucalyptol, 2-methyl-1-butanol, (*E*)-2-hexenal, ethyl hexanoate, octanal, 1-octen-3-ol, nonanal, (*E*)-2-octenal, ethyl (methylthio)acetate, ethyl (methylthio)acetate, 2,6-dimethyl-5-heptenal, hexyl acetate, 2-ethyl-2-hexenal, ethyl hexadecanoate, citronellal, benzyl alcohol, (*E*,*E*)-2,4-ethylhexadienoate, (*E*,*E*)-2,4-heptadienal, decanal, 1-octanol, ethyl-3 (methylthio)propionate, (*E*)-2-nonenal, 2,3-butanediol, β-cyclocitral, phenylacetaldehyde, ethyl decanoate, ethyl benzoate, 1-chlorodecane, 1-nonanol, α-terpineol, 1-decanol, α-farnesene, ethyl phenylacetate, α-isomethyl-ionone, β-ionone, β-ionol, 2-phenyl-2-butenal, 1-dodecanol, cinnamaldehyde, 3-phenylpropanol, octanoic acid, nonanoic acid, farnesylacetone, nootkatone (internal standard), *n*-alkane standards (C_5–C_{24}), SPME fibers, glucose, sucrose, and fructose were obtained from Sigma-

Aldrich (St. Louis, MO, USA). Sodium chloride (Fisher Scientific, Pittsburg, PA USA) was added to improve the extraction of volatile compounds.

Color analysis

The color of the fruit samples was measured using a Minolta CR-400 Chroma Meter (Konica Minolta Sensing, Inc., Osaka, Japan). The instrument was first calibrated using a white tile standard calibration plate (Calibration Plate CR-A43, Minolta Cameras, Osaka, Japan). L* (0, black to 100, white), a* (-60, green to +60, red) and b* (-60, blue to +60, yellow) were measured for all the samples.

Sugar sample preparation and measurement using HPLC

Five grams of the pureed sample was put in a 50-mL centrifuge tube and 5 mL of nanopure water from the NANOPure system (Barnstead/Thermolyne, Dubuque, IA, USA) was added. The sample mixture was then homogenized at 7000 rpm (850 Homogenizer, Thermo Fisher Scientific, Waltham, USA) for 30 sec and then subjected to sonication for 30 min. The tubes were then centrifuged at 10,000 x *g* for 15 min after which the supernatant was transferred into 15-mL tubes. Then, 900 µL of the decanted solution was mixed with 300 µL of methanol in microfuge tubes and centrifuged again (8000 x *g* for 5 min) to get a clear solution. Each breeding line sample was prepared in triplicate and the final mixture was stored at -20 °C until analysis.

For analysis, 20 µL of the sample solution was injected into the HPLC system consisting of a binary pump, autosampler, refractive index detector (Perkin Elmer LC 200 Series, Norwalk, Conn., USA), and Reze x RCM-Monosaccharide Ca^{+2} (300 × 7.8 mm) column with a guard column Carbo-Ca (4×3 mm ID) (Phenomenex, Inc. Torrance, CA, USA). Nanopure water was

used as a mobile phase with a flow rate of 0.6 mL/min, while the column temperature was maintained at 80 °C. Standard curves for fructose, glucose, and sucrose were used for calculating the sugar contents.

Sample preparation for HS-SPME-GC-MS

Cantaloupes were longitudinally cut into four halves; the seeds were then removed, and the flesh was separated from the rind using a knife. The flesh from each fruit was then cut into small cubes and blended in a high-speed blender (Oster, Milwaukee, WI, USA) for 1 min to form a puree. One gram of puree from each sample was then weighed into a 20-mL headspace vial, to which 1 mL NaCl (30%, w/v) and 5 µL of nootkatone (0.025%, v/v) were added as an internal standard. Each sample was prepared in triplicate and stored at -20 °C until GC-MS analysis.

HS-SPME-GC-MS analysis

Melon samples were kept at room temperature for 30 min and then loaded into a TriPlus RSH auto-sampler (Austin, TX, USA). The volatile compounds were extracted using HS-SPME with a 50/30 µm carboxen/polydimethylsiloxane/divinylbenzene (CAR/PDMS/DVB) fiber. The extraction and the desorption time using SPME fibers were 30 min and 2 min respectively, at 80 °C, with constant agitation for 10 sec every 2 min. Following adsorption, the SPME fiber was injected into the GC injector at 225 °C. Helium gas was used as a carrier gas with a constant flow rate of 1 mL/min in splitless mode. Volatile analysis of the samples was performed using the Thermo Finnegan gas chromatogram coupled with Dual-Stage Quadrupole (DSQII) mass spectrometer (Thermo Fisher Scientific, Inc., San Jose, CA, USA; Thermo Fisher, Austin, TX, USA). Restek Rtx-Wax column (30 m x 0.25 mm id with 0.25 µm film thickness; Restek Corp., Bellefonte, PA, USA) was used for analysis. The initial oven temperature was held at 40 °C for 2

min and then increased to 210 °C at a rate of 5 °C/min, with a total run time of 37 min. The MS detector operated in the electronic ionization mode (70 eV), in a scan mode from 30 to 300 amu at a rate of 11.5 scans/sec. The mass transfer line and ion source temperature were maintained at 280 and 285 °C, respectively.

An additional positive ionization step was carried out with methane as a reagent gas at a flow rate of 1 mL/min. The mass transfer line and ion source temperature were 230 °C and 180 °C, respectively.

Identification and quantification of volatile compounds

The data was processed using Xcalibur software (v. 2.0.7, Thermo Fisher Scientific, Inc, San Jose, CA, USA). Volatile compounds were identified by comparing their Kovats indices (KI), mass spectra, and retention times to their respective standards. KI values were calculated under the same conditions as the samples, by calculating the retention times of *n*-alkane standards (C_5-C_{24}). Identification of the VOCs was based on comparing the sample mass spectra with NIST 05 Mass Spectral Database (NIST, Maryland) and Wiley 8 library. Nootkatone was used as an internal standard to quantify volatile compounds. The results were expressed as μg/kg fresh weight of the sample.

Statistical analysis

Analysis of each experiment was carried out in triplicate and all data were represented as the mean ± SD. The principal component analysis (PCA) was performed using the mean data with SIMCA 16.0.2 statistical software (Umetrics Inc., San Jose, CA, USA). All the data were normalized using log transformation to have a normal distribution.

RESULTS AND DISCUSSION

Color measurement

The color attribute values of the cantaloupe fruit from different breeding lines are shown in Table 1. The L* (lightness) values ranged from 53.43 to 73.89; the a* (green to red) values were all in the positive range (15.38 to 27.08) showing that the melons tended towards a reddish hue due to the presence of carotenoids. A similar pattern was observed for b* (blue to yellow) values,

Table 1. Color attributes L* (lightness), b* (blue to yellow), a* (green to red) in 28 melon breeding lines

Name	L*	b*	a*
BL-1	73.89	46.57	21.02
BL-4	59.94	41.02	19.18
BL-6	73.11	39.21	20.56
BL-7	59.46	39.29	17.65
BL-8	73.57	45.06	16.37
BL-9	54.6	40.69	23.3
BL-11	62.48	40.73	17.75
BL-12	62.76	42.58	20.64
BL-14	70.55	47.7	20.85
BL-15	57.58	41.26	20.53
BL-17	67.17	45.54	22.43
BL-18	63.03	42.89	21.35
BL-19	67.26	49.06	27.08
BL-20	72.96	46.12	20.48
BL-21	66.64	44.88	20.61
BL-22	68.13	44.59	18.71
BL-24	66.06	46.11	22.01
BL-25	53.43	37.59	20.86
BL-26	56.45	40.37	17.04
BL-28	64.04	41.71	18.31
BL-30	62.55	41.21	20.12
BL-33	68.96	34.82	15.38
BL-34	64.51	40.48	17.07
BL-35	56.97	43.3	21.71
BL-36	60.62	42.4	20.33
BL-40	55.16	41.67	19.45
BL-43	66.5	44.09	20.24
BL-100 (10)	72.93	48.44	18.25

where all the values were positive, ranging from 34.82 to 49.06 due to the presence of β-carotene.[50] Consumers judge a food product by its color, taste, and aroma; therefore, the color of fresh produce is one of the most important factors for consumer preferences.[49]

Sugar analysis

One of the major quality determinants in melon fruits is its sugar content and sucrose is the most abundant sugar in melons (~97%), followed by fructose and glucose.[28] The contents of the individual sugars along with total sugar content are shown in Table 2.

Table 2. Sucrose, glucose, fructose, and total sugar concentration (mg/g FW) in 28 melon breeding lines

Name	Sucrose	Glucose	Fructose	Total Sugars
BL-1	10.10 ± 0.52	4.15 ± 0.81	4.91 ± 1.14	19.17 ± 0.75
BL-4	28.09 ± 0.79	5.97 ± 0.21	16.10 ± 0.36	50.17 ± 1.25
BL-6	14.86 ± 10.55	9.05 ± 2.87	14.02 ± 1.94	37.94 ± 4.59
BL-7	19.86 ± 0.88	5.19 ± 1.51	13.21 ± 1.97	38.27 ± 1.91
BL-8	17.45 ± 3.49	6.59 ± 0.05	10.03 ± 3.22	34.07 ± 3.01
BL-9	21.14 ± 0.83	7.42 ± 0.11	14.36 ± 0.4	42.93 ± 1.34
BL-11	16.41 ± 5.03	4.57 ± 1.03	11.11 ± 1.98	32.10 ± 3.65
BL-12	23.61 ± 10.53	5.20 ± 1.32	12.48 ± 3.73	41.3 ± 7.28
BL-14	25.43 ± 0.06	9.32 ± 0.04	18.93 ± 0.86	53.69 ± 0.83
BL-15	14.23 ± 0.33	3.59 ± 0.06	9.78 ± 0.03	27.61 ± 0.42
BL-17	15.40 ± 0.71	11.33 ± 0.4	24.01 ± 0.68	50.75 ± 1.76
BL-18	7.55 ± 4.43	3.49 ± 1.21	8.79 ± 2.6	19.84 ± 4.17
BL-19	12.18 ± 0.92	4.77 ± 0.36	12.21 ± 0.75	29.17 ± 2.04
BL-20	12.08 ± 10.73	5.80 ± 1.85	6.55 ± 0.14	24.43 ± 3.9
BL-21	19.34 ± 0.19	3.43 ± 0.06	4.42 ± 0.15	27.20 ± 0.28
BL-22	23.67 ± 0.72	9.91 ± 0.22	21.94 ± 0.94	55.54 ± 1.87
BL-24	26.88 ± 1.69	5.63 ± 0.25	14.46 ± 0.76	46.98 ± 2.67
BL-25	20.14 ± 4.51	6.17 ± 1.94	14.55 ± 4.34	40.87 ± 4.93
BL-26	14.07 ± 0.38	6.29 ± 0.13	16.57 ± 0.44	36.93 ± 0.95
BL-28	16.91 ± 11.22	8.29 ± 5.09	20.87 ± 8.75	46.08 ± 1.26
BL-30	2.68 ± 2.11	6.22 ± 2.96	10.93 ± 1.95	19.84 ± 2.00
BL-33	20.55 ± 2.99	4.71 ± 1.9	11.99 ± 3.81	37.27 ± 4.14
BL-34	10.65 ± 0.81	5.43 ± 1.28	7.53 ± 2.05	23.61 ± 1.43
BL-35	13.72 ± 2.9	5.23 ± 1.19	5.94 ± 1.4	24.90 ± 1.22
BL-40	18.98 ± 0.63	3.39 ± 0.2	4.81 ± 0.05	27.20 ± 0.47
BL-43	18.50 ± 0.77	5.39 ± 0.16	7.07 ± 0.13	30.97 ± 1.06

Sucrose contents ranged from 2.68–28.09 mg/g FW with the highest in BL-4 (28.09 mg/g FW) followed by BL-24 (26.88 mg/g FW). The fructose and glucose contents ranged from 4.42–24.01 mg/g FW and 3.39–11.33 mg/g FW, respectively, and BL-17 had the highest contents of these sugars. The total sugars ranged between 19.17–55.54 mg/g FW, with the highest content in BL-22 followed by BL-14.

Identification and quantification of volatile compounds

The HS-SPME-GC-MS analysis identified a wide range of VOCs from the 28 melon breeding lines as shown in Table 3. The 113 VOCs detected in the 28 melon lines included 27 aldehydes, 15 esters, 35 alcohols, 14 ketones, 10 hydrocarbons, 4 acids, and 5 sulfur-containing VOCs. The other 3 volatiles identified were benzeneacetonitrile, 2-pentylfuran, and methoxy-phenyl-oxime. A typical chromatograph representing the melon VOCs in BL-20 is shown in Figure 1. The highest total VOC content was found in BL-30 (13973.07 µg/kg FW) whereas, the lowest was found in BL-22 (3947.13 µg/kg FW); BL-20 had the most different VOCs (77), and BL-22 had the fewest different VOCs (42).

Table 3. Concentration ranges of volatile compounds recovered from 28 melon breeding lines via HS-SPME GC-MS harvested in the year 2019. The volatile compounds were identified by comparing the mass spectra and Kovats indices (KI)

RT	**Compounds**	**KI**	**Concentration range (mg/kg)**
7.55	limonene	1035	0-57.27
7.58	eucalyptol	1198	0-34.49
8.35	2-methyl-1-butanol	1206	0-222.36
8.38	(*E*)-2-hexenal	1220	0-151.66
8.56	2-pentylfuran	1231	0-83.7
8.75	ethyl hexanoate	1234	0-548.93
9.08	*trans*-α-ocimene	1237	0-36.22

Table 3. Continued

RT	Compounds	KI	Concentration range (mg/kg)
9.49	1-pentanol	1252	0-6.25
9.75	hexyl acetate	1275	0-182.4
10.19	octanal	1286	0-320.98
10.19	3-hydroxybutan-2-one	1289	0-429.21
10.78	2,6-dimethyl-5-heptenal	1315	0-5.02
10.98	(*Z*)-2-heptenal	1323	0-24.34
11	(4*E*)-4-hexenyl acetate	1326	0-182.77
11.31	2-ethyl-2-hexenal	1330	0-333.96
11.45	6-methyl-5-hepten-2-one	1341	0-43.11
12.04	1-hexanol	1360	0-365.47
12.35	dimethyl trisulfide	1383	0-19.31
12.83	(*Z*)-3-hexen-1-ol	1386	0-366.45
12.84	nonanal	1396	0-162.94
13	(2*E*,4*E*)-hexadienal	1400	0-41.47
13.7	(*E*)-2-octenal	1432	3.45-94.91
13.95	(*E*)-4-nonenal	1435	0-9.54
13.95	ethyl caprylate	1440	0-95.43
14.04	3,7-dimethyloctan-3-ol	-	0-22.54
14.25	ethyl (methylthio)acetate	1452	0-293.87
14.25	(*E*)-6-nonenal	1453	0-132.62
14.45	1-octen-3-ol	1456	0-231.06
14.45	acetic acid	1480	0-194.67
14.95	citronellal	1495	0-29.95
15.11	ethyl 2,4-hexadienoate	1501	0-27.85
15.28	(*E*,*E*)-2,4-heptadienal	1506	0-158.44
15.34	methyl nonanoate	1515	0-15.39
15.46	decanal	1521	22.45-62.45
15.95	benzaldehyde	1530	134.35-4795.74
16.3	(*E*)-2-nonenal	1543	60.09-1039.43
16.44	ethyl nonanoate	1548	0-26.49
16.67	2,3-butanediol	1550	0-17.28
16.79	linalool	1552	0-4.66
17.08	1-octanol	1561	0-468.01
17.15	3,5-octadien-2-one	1567	0-113.59
17.11	ethyl-3(methylthio)propionate	1571	0-112.05

Table 3. Continued

RT	Compounds	KI	Concentration range (mg/kg)
17.5	(*E*,*Z*)-2,6-nonadienal	1596	31.89-2028.31
17.65	3-octen-1-ol	1563	0-453.78
17.85	hexadecane	1600	0-116.33
17.97	terpinen-4-ol	1612	0-12.98
17.97	isopulegol	1606	0-14.51
18.24	β-cyclocitral	1623	63.31-308.21
18.55	octyl-2-methylbutanoate	1634	0-16.34
18.55	3-(methylthio)propyl acetate	1633	0-70.41
18.7	phenylacetaldehyde	1640	11.4-121.98
18.81	ethyl decanoate	1642	0-45.74
18.93	4-methyl-5-decanol	-	0-104.14
18.93	isopinocarveol	1642	0-6.01
19.15	β-cedrene	1648	0-3.41
19.33	ethyl benzoate	1650	0-607.17
19.43	1-nonanol	1655	0-32.84
19.66	cis-verbenol	1663	0-11.18
19.95	(Z)-3-nonenol	1682	13.02-514.19
20.17	α-terpineol	1688	129.04-314.73
20.34	dodecanal	1710	18.45-93.15
20.67	3-(methylthio)propanol	1711	0-100.65
20.81	citral	1714	0-33.21
20.92	1,4-dimethoxybenzene	1728	0-6.04
21.2	α-farnesene	1747	0-25.56
21.29	δ-cadinene	1748	0-15.81
21.39	(*E*,*Z*)-3,6-nonadien-1-ol	1749	0-416.92
21.69	1-decanol	1760	0-77.79
21.75	3-phenylpropanal	1783	0-62.85
21.88	methoxy-phenyl-oxime	-	21.7-84.66
22	ethyl phenylacetate	1786	0-79.32
22.14	3-decen-1-ol	1790	0-1070.61
22.44	1-phenyl-1,2-propanedione	1818	0-155.53
22.47	(*E*,*E*)-2,4-decadienal	1826	0-40.46
23.16	carveol	1836	17.27-56.98
23.27	ethyl dodecanoate	1840	0-10.11
23.27	α-isomethyl-ionone	1848	0-27.22

Table 3. Continued

RT	Compounds	KI	Concentration range (mg/kg)
23.31	α-ionone	1849	0-22.84
23.38	hexanoic acid	1854	0-51.87
23.48	geranylacetone	1865	0-3152.62
23.98	benzyl alcohol	1880	13.75-1067.05
24.59	α-calacorene	1920	7.45-132.19
24.67	2-phenylethanol	1915	0-238.75
24.82	tetradecanal	1919	13.49-122.04
24.83	benzeneacetonitrile	1931	0-28.92
24.92	2-phenyl-2-butenal	1932	0-549.66
25.12	β-ionone	1947	198.54-1568.57
25.39	benzothiazole	1948	13.52-51.46
25.54	β-ionol	1968	0-62.15
25.67	3-phenyl-2-butenal	-	0-125.5
25.9	1-dodecanol	1970	0-133.89
26.15	β-ionone epoxide	1977	81.43-673.58
26.27	α-methylcinnamaldehyde	1992	0-26.87
26.82	γ-nonalactone	2018	0-141.3
27.02	cinnamaldehyde	2025	0-305.08
27.32	3-phenylpropanol	2058	4.59-1823.52
27.65	octanoic acid	2070	0-165.72
27.81	globulol	2085	0-14.38
27.93	elemol	2090	61.95-120
28.63	cedrenol	2110	0-29.76
29	γ-decalactone	2144	0-236.55
29.53	eugenol	2162	0-21.77
29.58	*T*-cadinol	2165	0-57.1
29.62	nonanoic acid	2169	0-349.88
29.77	1-tetradecanol	2174	0-319.76
30.12	δ-cadinol	2179	15.53-31.85
30.46	methyl hexadecanoate	2202	0-125.98
30.67	α-cadinol	2217	0-72.28
30.93	methyl 9-hexadecanoate	2278	0-275.22
31.12	ethyl hexadecanoate	2288	13.76-299.48
32.38	dihydroactinidiolide	2291	45.89-342.77
33.12	farnesyl acetone	2363	17.89-156.17

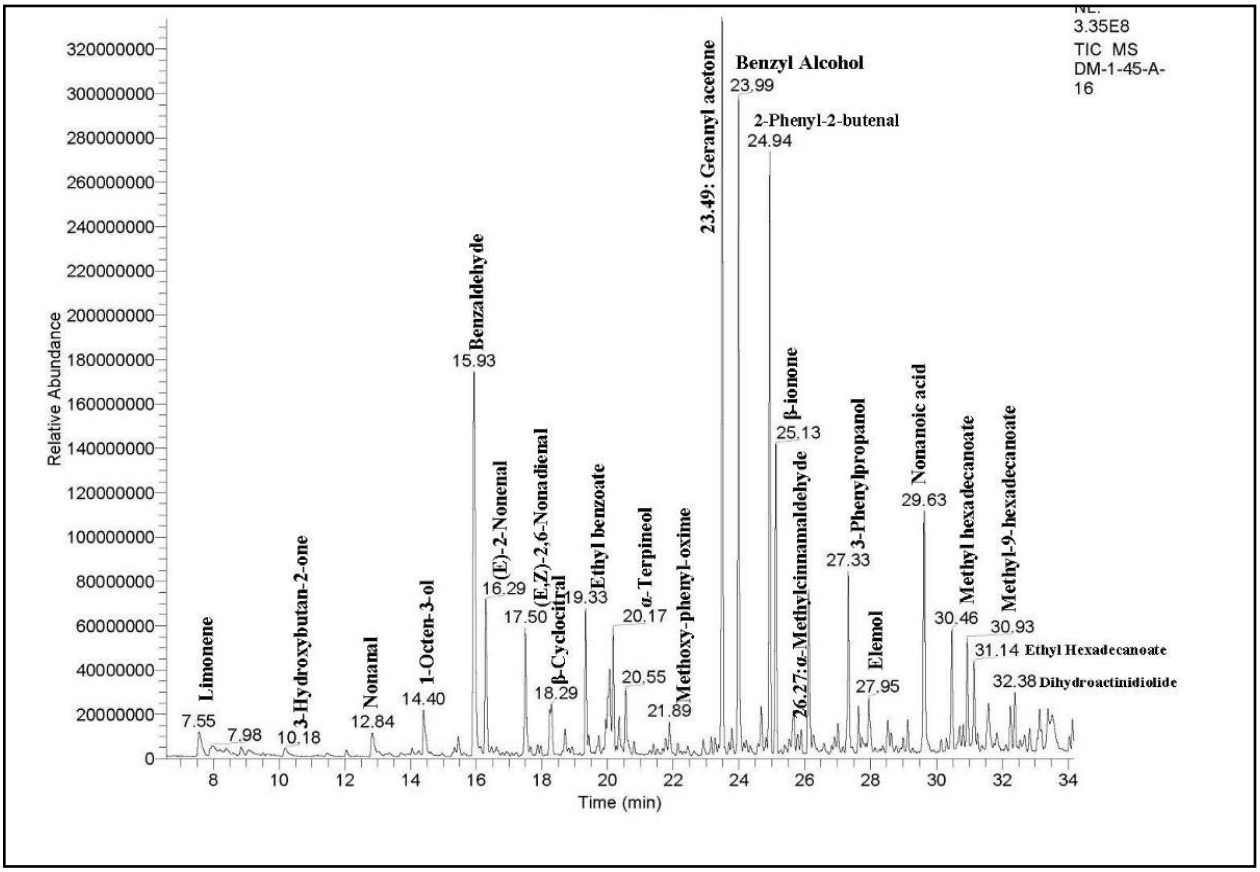

Figure 1. GC-MS profile of the volatile fraction of melon breeding line BL-20, using HS-SPME/GC-MS

Esters

Our breeding lines are all climacteric and, consistent with that, we identified fifteen esters in the cantaloupe breeding lines, with the total average volatile content of 3.78% in the 28 lines, shown in Table 4. BL-26 (1227.84 µg/kg FW) had the highest total average content whereas BL-22 (22.74 µg/kg FW) had the lowest. The ester present in the highest average content was ethyl benzoate (96.42 µg/kg FW) in BL-28 and BL-21. BL-26 and BL-28 showed high ethyl hexanoate levels. In this study, ethyl hexadecanoate was identified in all the lines. Ethyl hexadecanoate is known for its waxy flavor and aroma while ethyl benzoate and ethyl hexanoate are known for their

fruity and musty aroma coupled with a minty flavor.[52, 53] Esters are major aroma compounds responsible for fruity notes and flavor characteristics in many fruits and vegetables.[13, 14] Moreover, previous studies have demonstrated that climacteric varieties of melons appear to have high amounts of esters whereas non-climacteric varieties lack esters and have comparatively low amounts of total aroma compounds.[13, 48, 51]

Aldehydes

The 27 aldehydes identified here made up 37.16% of the total VOC composition as shown in Table 4. The highest aldehyde content was found in BL-12 (6167.67 µg/kg FW), followed by BL-4 (5966.35 µg/kg FW); these lines exhibited high benzaldehyde contents. Benzaldehyde, (*E*)-2-nonenal, and (*E,Z*)-2,6-nonadienal were present in all the lines. BL-34 had the highest (*E,Z*)-2,6-nonadienal and (*E*)-2-nonenal contents (2028.31 µg/kg and 1039.43 µg/kg FW). Benzaldehyde is associated with an almond-like odor and flavor,[54] whereas (*E*)-2-nonenal and (*E,Z*)-2,6-nonadienal exhibit strong waxy and cucumber-like flavors, respectively.[55]

Alcohols

Thirty-five alcohol compounds, contributing to 10.40–49.96% of the total VOC content were found in the lines (Table 4). High alcohol contents were found in BL-30 (6981.21 µg/kg FW) followed by BL-26 (3566.96 µg/kg FW). Benzyl alcohol (499.32 µg/kg FW) and 3-phenylpropanol (271.79 µg/kg FW) were the most abundant alcohol VOCs, indicating their importance in the melon aroma. Indeed, (*E,Z*)-3,6-nonadien-1-ol and benzyl alcohol are known to be major constituents of melon aroma.[14] BL-26 had the highest contents of benzyl alcohol and 3-phenylpropanol was the highest in BL-30. The lowest benzyl alcohol and 3-phenylpropanol

content were observed in BL-22. Moreover, benzyl alcohol, 3-phenylpropanol, and α-terpineol were present in all the lines. A high level of α-terpineol (>260 µg/kg FW), was present in BL-30 and BL-7. Similarly, (*E,Z*)-3,6-nonadien-1-ol (>400 µg/kg FW) was found in BL-36 and BL-40.

Ketones

Fourteen ketones, which accounted for 13.84–44.96% (Table 4) of the total VOC content, were identified. BL-26 had the highest content (4583.84 µg/kg FW) of all ketones followed by BL-9 (4463.78 µg/kg FW) whereas BL-33 showed the lowest total ketones (976.60 µg/kg FW). Geranylacetone and β-ionone showed the highest average contents (806.53 and 652.17 µg/kg FW) and were higher in BL-26 and BL-9 than in other lines. Geranylacetone and β-ionone are known for their fruity, tropical aroma and flavor.[56] Neryl acetone, an isomer of geranylacetone, has not been previously reported in melons, to the best of our knowledge. β-Ionone, 6-methyl-5-hepten-2-one, β-ionone-epoxide, dihydroactinidiolide, and farnesyl acetone were the 5 ketones present in all the breeding lines.

Acids

In the present study, we identified four acidic VOCs: acetic acid, hexanoic acid, octanoic acid, and nonanoic acid, which accounted for an average of 0.94% of the total VOC contents in six of the lines (Table 4). BL-6 had the highest level (443.50 µg/kg FW) of acids and BL-19 had the lowest (5.10 µg/kg FW). Nonanoic acid showed the highest average content (40.29 µg/kg FW) followed by octanoic acid (21.12 µg/kg FW); BL-20 had high nonanoic acid contents and BL-6 had high octanoic acid contents.

Short and medium chain fatty acids such as hexanoic (C6:0) and octanoic (C8:0) acids have a wide spectrum of antimicrobial effects against bacteria, viruses, and fungi.[57, 58] The present levels were in accordance with a previous study[59] which reported the fatty acids hexanoic acid (1.81%), octanoic acid (0.99%), and nonanoic acid (1.82%) in melons. Octanoic acid is naturally present in milk, coconut oil, and fruits and vegetables,[60, 61] and has generally recognized as safe (GRAS) status.[62] Octanoic acid has a significant antimicrobial effect against *L. monocytogenes*, *Salmonella spp*., and *Escherichia coli* O157:H7 on various fresh produce such as spinach, grape tomatoes, and cantaloupe rind.[63, 64, 65] Moreover, fatty acids that are naturally present on tomato surfaces inhibit the growth of *Salmonella enterica*.[66] It is possible that increasing the levels of these acids in selected melons, along with other quality parameters would be useful for consumer safety.

Hydrocarbons

An average of 1.18% of the total VOC content represented ten hydrocarbon compounds (Table 4). BL-35 had the highest content of hydrocarbons (206.39 µg/kg FW) among all the lines, and BL-43 had the lowest (41.41 µg/kg FW). The compounds, benzothiazole and α-calacorene were present in all the lines with 26.17 and 20.52 µg/kg FW average contents, respectively; the highest benzothiazole level was identified in BL-4 (51.46 µg/kg FW) and the lowest in BL-24 (13.52 µg/kg FW). Moreover, the highest α-calacorene level was found in BL-36 (132.19 µg/kg FW) and the lowest was observed in BL-40 (7.45 µg/kg FW). While these compounds do not have particularly good aromatic properties, benzothiazole has anti-tumor and anti-microbial properties.[67]

The hydrocarbon D-limonene was found in all the lines (~82%) except BL-24, BL-26, BL-30, and BL-43. The highest D-limonene concentration was found in BL-20 (57.27 µg/kg FW).

Limonene has significant health beneficial properties such as an anti-proliferative effect, prevention of gastric diseases, and anticancer activity, along with a lemony aroma.[68-71] Furthermore, D-limonene is a potent antimicrobial compound that inhibits the growth of foodborne pathogens such as *L. monocytogenes* and *Salmonella spp*.[72, 73]

Sulfurs

Five sulfur-containing VOCs were identified in this study. BL-28 showed the highest contents (516.20 µg/kg FW) and BL-4 had the lowest (4.25 µg/kg FW). Ethyl (methylthio)acetate was present in the highest average content (25.07 µg/kg FW) and has a major effect on the musky note of melon aroma.[74]

Others

Benzeneacetonitrile, 2-pentylfuran, and methoxy-phenyl-oxime made up 0.76% of the total VOCs (Table 4). To the best of our knowledge, methoxy-phenyl-oxime has only been previously reported in one other published study on melon.[75] Based on information from The Good Scents Company, benzeneacetonitrile and 2-pentylfuran have good aromatic properties. BL-35 had the highest content (113.58 µg/kg FW), whereas BL-8 had the lowest average content (35.77 µg/kg FW). BL-35 also had the highest methoxy-phenyl-oxime content (84.66 µg/kg FW).

Table 4. Total % of each type of volatiles in 28 melon breeding lines

BL	Esters	Aldehydes	Alcohols	Ketones	Acids	Hydrocarbons	Sulfurs	Others
BL-1	4.82	45.40	25.20	19.31	2.83	0.84	0.62	0.97
BL-4	0.54	62.53	10.40	24.86	0.00	1.18	0.04	0.44
BL-6	5.41	47.11	26.49	13.84	4.97	1.03	0.49	0.66
BL-7	1.35	46.74	25.14	24.49	0.19	1.04	0.49	0.57
BL-8	1.79	40.05	30.15	26.00	0.36	0.96	0.11	0.58
BL-9	2.36	44.70	12.38	39.37	0.00	0.49	0.07	0.64
BL-11	3.22	33.28	26.27	32.60	1.45	1.33	1.12	0.73
BL-12	1.10	60.45	12.88	24.20	0.14	0.51	0.24	0.47
BL-14	1.13	28.73	28.39	38.95	0.19	1.36	0.09	1.16
BL-15	4.81	30.02	27.07	35.80	0.00	0.93	0.73	0.64
BL-17	1.93	49.98	20.88	24.25	0.13	1.09	1.20	0.52
BL-18	5.06	43.18	25.02	23.89	0.48	0.92	0.87	0.59
BL-19	1.20	37.82	14.03	44.96	0.07	0.72	0.28	0.93
BL-20	8.85	36.25	22.88	24.46	3.91	1.90	1.18	0.58
BL-21	9.95	30.98	28.33	27.13	0.16	1.49	1.04	0.92
BL-22	0.58	39.50	20.33	36.51	0.00	1.72	0.28	1.08
BL-24	6.15	33.48	24.98	32.88	0.00	0.77	0.40	1.34
BL-25	1.35	36.43	18.68	41.27	0.00	1.38	0.10	0.79
BL-26	9.10	29.09	26.45	33.99	0.17	0.76	0.06	0.37
BL-28	11.23	28.33	35.11	16.33	0.32	2.08	6.10	0.50
BL-30	2.19	25.19	49.96	19.08	2.13	0.70	0.00	0.75
BL-33	1.78	33.58	45.31	16.20	0.71	1.42	0.21	0.79
BL-34	3.16	48.33	15.06	30.18	0.64	0.67	1.13	0.84
BL-35	1.73	37.33	24.09	31.64	1.20	2.21	0.57	1.22
BL-36	1.89	21.22	42.42	30.97	0.12	2.42	0.22	0.74
BL-40	1.85	19.41	48.95	27.09	0.41	1.14	0.15	1.01
BL-43	10.66	35.08	20.44	27.49	5.15	0.60	0.00	0.58
BL-100(10)	0.74	16.36	35.84	43.64	0.58	1.41	0.70	0.73
Total	**3.78**	**37.16**	**26.54**	**28.98**	**0.94**	**1.18**	**0.66**	**0.76**

VOCs with antimicrobial properties in selected lines

The use of VOCs to extend the shelf life of food products has received substantial attention in recent years as an alternative to chemical preservatives.[15, 11] One of the major objectives of our project was to enable the production of safer melons along with enhanced fruit and nutritional quality, flavor, and stress resistance. Several melon lines produced using genomics-assisted breeding were screened for the aforementioned attributes, including the 28 breeding lines reported in this study. Of note, our ongoing study indicates some of these lines have high levels of antimicrobials such as D-limonene, nonanoic acid, benzaldehyde, geranylacetone, and α-terpineol,

which may reduce the overall persistence of foodborne pathogens such as *L. monocytogenes* and *Salmonella*.

In addition, many of the lines in this study produce fruit with smooth netted surfaces. As observed in our previous study,[76] lightly netted rind surfaces have lower levels of microbial attachment. As also observed by Vitha (2021),[77] *Salmonella* showed a high attachment strength on densely netted cantaloupe rinds, followed by medium netted and lightly netted rinds. Other factors such as attachment, transfer rates, dust, and contamination routes from contact surfaces also affect bacterial levels and susceptibility to the antimicrobials present on the rind surface.[78] Therefore, the VOCs identified in our melon breeding lines will be studied as potential biomarkers for their association with bacteria levels to improve food safety. The VOCs identified in specific lines and their antimicrobial efficacy are briefly discussed below.

BL-30 had a high average level of VOCs such as geranylacetone and 3-phenylpropanol, which contribute to the flavor and aromatic properties of various fruits.[56] Geranylacetone was also isolated from the horsetail *Equisetum arvense L*. and its essential oil form inhibited *Salmonella*.[79] Analogs of geranylacetone were tested for antimicrobial activities against *Staphylococcus aureus*, *Enterococcus*, *E. coli*, and *Klebsiella spp*.[80, 81] BL-30 had a high content of α-terpineol, which is usually found in citrus, tea tree, beer, and coffee, and is known for its antimicrobial properties against *E. coli*,[82] *L. monocytogenes*, *S. aureus*, and *Bacillus cereus*.[15, 17]

BL-20 had the highest number of total volatiles along with a high benzaldehyde content (1628.10 µg/kg FW). Benzaldehyde exhibits a strong antimicrobial effect against *L. monocytogenes* under anaerobic conditions.[83] The highest antimicrobial activity of benzaldehyde with surface sulfhydryl groups was on *Salmonella* followed by *Listeria* and *Lactobacillus*.[83] Benzaldehyde in the form of essential oil at 8–10 µL/mL, and as a polymer, inhibited foodborne

pathogens in different fruit juices and broth.[84, 85] BL-20 also had the highest contents of linalool (4.66 µg/kg FW), a compound known for its antimicrobial effect against *Salmonella spp.*, *L. monocytogenes*, *E. coli* and *S. aureus*.[16, 86]

Nonanoic acid was the highest in BL-20 (349.88 µg/kg FW). Nonanoic acid is well known in the food industry for its antimicrobial and antifungal properties. It is used as a textile coating and inhibits the growth of *E. coli* and *S. aureus*.[87] Nonanoic acid, in an emulsion form, also proved to be more effective than traditional sanitizers against *Salmonella* growth on tomatoes during post-harvest storage.[88]

BL-7 had high sucrose and total sugar contents and had the highest contents of eucalyptol (34.49 µg/kg FW), which is known for its antimicrobial effect against *E. coli* and *S. aureus*.[89] BL-4 had high sucrose contents and was rich in β-ionone, α-terpineol, geranylacetone, benzaldehyde, and D-limonene. BL-12 had the highest benzaldehyde contents (4795.74 µg/kg FW), a high sucrose content, and high levels of good aromatic and flavorful compounds such as geranylacetone and β-ionone. The breeding lines BL-30, BL-20, BL-7, BL-4, and BL-12, as described, have good sensory properties, compounds with good antimicrobial activities, as well as high amounts of sugars. Additionally, BL-9 had a high a* value (Table 1) along with high levels of β-ionone (1568.57 µg/kg FW), dihydroactinidiolide (342.77 µg/kg FW), β-cyclocitral (308.21 µg/kg FW), α-ionone (22.84 µg/kg FW), and 2,6-dimethyl-5-heptenal (5.02 µg/kg FW); these compounds are associated with high lycopene and β-carotene contents in watermelons[90] and produce an orange hue, thus making the fruit visually appealing to the consumers. Carotenoids are also known to be beneficial to human health, as consuming a carotenoid-rich diet can reduce the risk of cancer, cardiovascular diseases, macular degeneration, cataracts, and UV-induced skin damage.[91, 92]

Principal component analysis (PCA)

Principal component analysis (PCA) was used as a multivariate tool to detect correlations between all the breeding lines and VOCs. PC1 and PC2 accounted for 17.2% and 15.3% of the total variation, respectively (Figure 2A). In the loadings plot (Figure 2B), most of the alcohols, aldehydes, and esters are on the right side of the PC1 axis, making up the 1st and 4th quadrants. The breeding lines correlating to these are BL-9, BL-7, BL-4, BL-20, BL-35, BL-12, BL-6, BL-1, BL-43, BL-28, BL-26, and BL-30. Since aldehydes, alcohols and esters are the chemical classes usually associated with melon aroma and flavor,[40, 41] this suggests that these lines produce fruit with good odor and flavor properties. Some of the major compounds present in these quadrants are eucalyptol, benzaldehyde, carveol, citral, geranylacetone, limonene, cinnamaldehyde, α-calacorene, benzothiazole, β-ionone, and benzyl alcohol. However, the 4th quadrant of the loadings plot (Figure 2B), indicates the presence of two sulfurous and four acidic compounds, which suggest an intricate relationship between sensory properties and breeding line. Considering the 2nd and 3rd quadrants, which lie on the left side of the PC1 axis (Figure 2), we found that only a few VOCs associate with the breeding lines present in these two quadrants (BL-19, BL-25, BL-22, BL-34, BL-24, BL-17, BL-14, BL-21, BL100 (10), BL-8, BL-18, BL-40, BL-36 and BL-33).

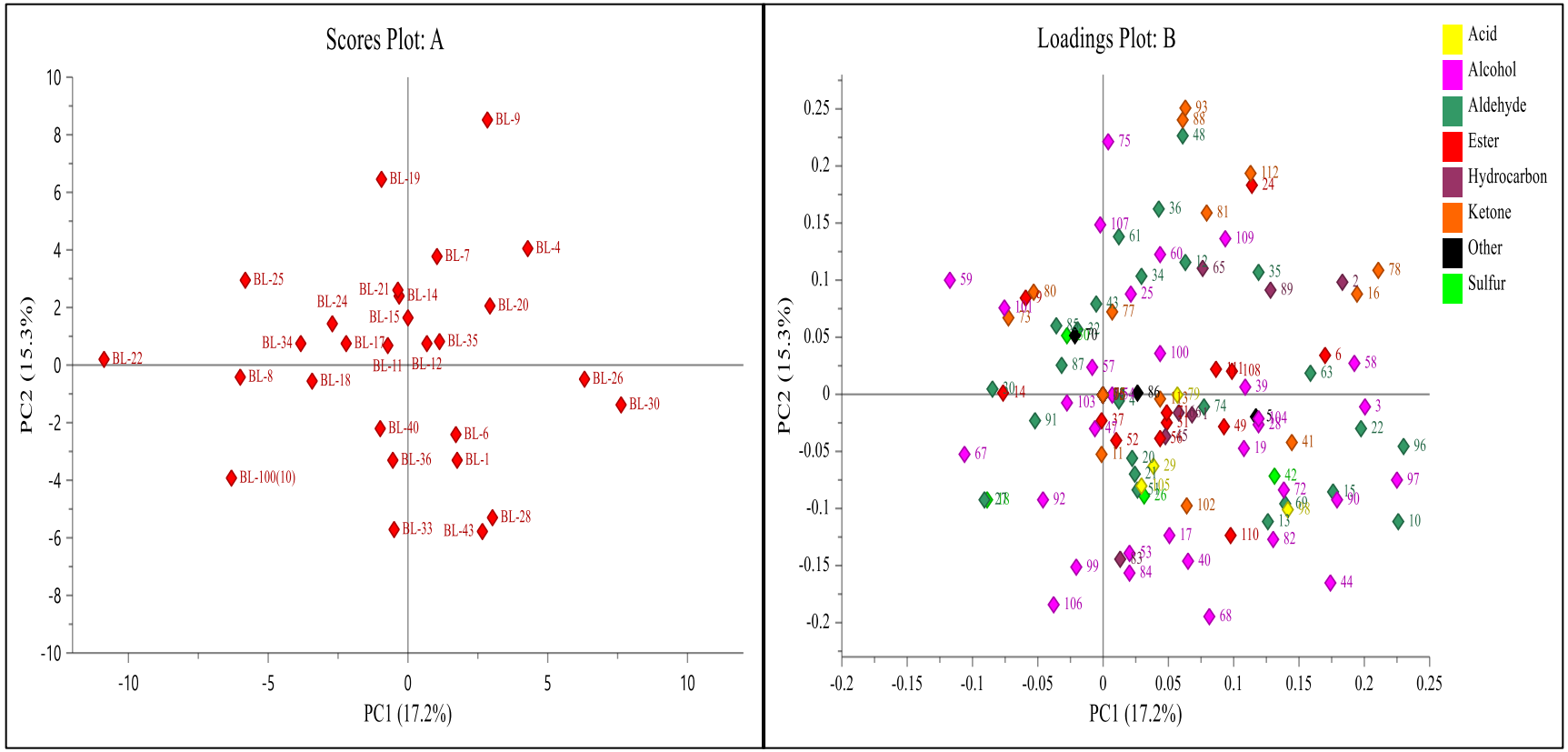

Figure 2. Principal component analysis of 28 melon breeding lines displayed according to their chemical classes. (A) corresponds to the scores plot. (B) corresponds to the loadings plot and the codes indicate the volatile compounds, as displayed in Table 3

Methoxy-phenyl-oxime was associated with these lines; this compound is known to have anti-bacterial properties[93] and has been reported to be present in bamboo shoots,[52, 53] but has only been reported in muskmelons once.[75] Further research on its sensory and/or antibacterial properties should therefore be conducted.

Among all the breeding lines, the highest average VOC content was observed in BL-30 (13973.07 µg/kg FW). 3-Phenylpropanol (1823.52 µg/kg FW), geranylacetone (1191.69 µg/kg FW) and 3-decen-1-ol (1070.61 µg/kg FW) were found in the highest quantities in BL-30 (average content > 1000 µg/kg FW). 3-Phenylpropanal is known for its fruity, spicy, and floral aroma,[94] and geranylacetone exhibits a fruity, tropical aroma and flavor and is also known for its anti-microbial properties.[80] The highest number of volatiles was observed in BL-20 (77), with benzaldehyde (1628.10 µg/kg FW) followed by geranylacetone (1223.77 µg/kg FW), exhibiting the highest average contents.

The lines characterized here had more aldehydes and alcohols than esters, which implies that the melons were not fully mature, since the presence of esters is mostly associated with ripe, mature melons whereas aldehydes are mostly associated with immature fruits.[51] This is in accordance with the color readings (Table 1), where BL-4, BL-7, BL-11, BL-15, BL-28, BL-30, BL-33, BL-34 and BL-40 associated with lightness and green color along with high contents of aldehydes and alcohols (Figure 2A, B).

BL-33, BL-7, BL-4, BL-24, BL-36, BL-9, BL-14, BL-25, BL-26, BL-6, BL-28, and BL-22 were positively associated with the three main sugars along with total sugars (Figure 3). Although BL-17 and BL-12 did not have total sugar values associated with them, BL-17 was positively correlated to glucose and fructose while BL-12 had more sucrose. Future breeding programs could focus on

selecting lines with good sensory properties i.e., high aroma and high sugar content, and with compounds having anti-microbial and health beneficial properties.

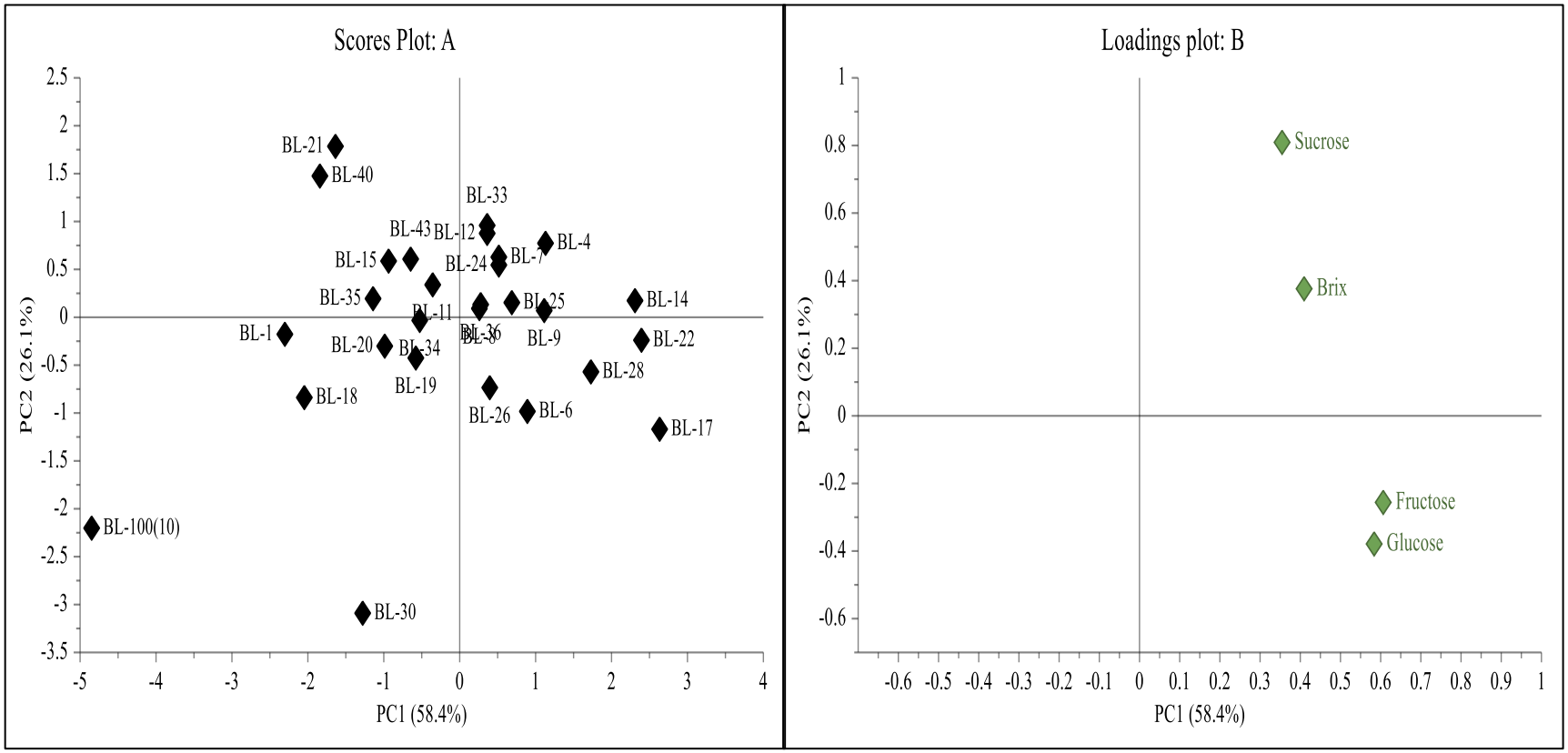

Figure 3. Principal component analysis displaying the breeding lines correlation with individual and total sugars. Figure 3A corresponds to the breeding lines and loadings plot; Figure 3B corresponds to the three sugars and total sugars

CHAPTER IV

CHARACTERIZATION OF VOLATILES, ANTIOXIDANT ACTIVITIES, SUGARS, PH AND CAROTENOID PROFILES IN *CUCUMIS MELO* L. BREEDING LINES GROWN IN TEXAS

INTRODUCTION

The global melon production increased by 1.6% from 2017, with 33 Million tons produced in 2018. The per capita consumption of melons in the United States was approximately 7 pounds in 2018.[95, 96] Volatile organic compounds (VOCs) are the major determinants and play a key role in the quality of melon as they contribute to the aroma of the fruit which results from a combination of volatile substances including esters, alcohols, aldehydes, ketones, sulfurs.[97] Melon sweetness is another important attribute associated with consumer preferences.[25, 26] Commonly, *Cucumis melo* L. fruits have high sugar levels with low citric acidity, however, mature fruits are sometimes also found to have a high acidic content, thereby making the taste of the fruit highly dependent on the combination of sugar/acid ratio.[98]

Important health-promoting bioactive compounds present in melon include ascorbic acid (vitamin C), folic acid and L-citrulline and other essential micronutrients.[26, 99, 100] β-carotene (vitamin A) is a major carotenoid found in the cantaloupe.[101, 102] These bioactive compounds including β-carotene present in melon contribute to health-promoting properties which can reduce the risk of degenerative diseases such as cancer, cardiovascular diseases, diabetes and age-related macular degeneration.[103] Some studies also showed an inverse relationship between cardiovascular diseases and consumption of carotenoid-rich diet including melons.[31, 91]

The presence of these compounds from melon has profound importance due to their biological and high antioxidant activity that has escalated the consumer interest. Antioxidants are

considered natural and free of harmful chemicals.[104] Antioxidants protect the cell from free radical damage by neutralizing and scavenging them.[19] Previous studies have investigated antioxidant and radical scavenging activities in melons by utilizing assays such as 2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH).[105] Increasing the level of bioactive compounds in fruits has been a major challenge for melon breeders.

The important breeding goals for melons are to improve their sustainability and sales in the US by mainly concentrating on consumer preferences which include overall quality with high sugar content, taste, rich in phytonutrients and bioactive compounds and long shelf life. Current knowledge on aforementioned qualities for breeding lines in melon remains limited. In light for improving the overall quality, our goals were to (a) characterize the volatile profiles, pH, citric acid activity, sugar content, and carotenoid contents and (b) determine the antioxidant and free radical scavenging properties from *Cucumis melo* L. breeding lines grown in the years 2018 and 2019, in Uvalde, Texas, USA. Head space solid-phase microextraction (HS-SPME) coupled with gas chromatography-mass spectrometry (GC-MS) was used for the identification of VOCs. pH, sugars, total phenolics, antioxidant and carotenoid profiles were determined for the breeding lines grown over the course of two years.

MATERIALS AND METHODS

Plant materials

In this study, 6 melon breeding lines were harvested in the year 2018 and 15 breeding lines in the year 2019 in Uvalde, Texas during the period of March to July. The fruits were then

transported to the Vegetable and Fruit Improvement Centre (VFIC), in College Station, Texas for further analysis.

Chemicals

Methanol, L-ascorbic acid and sodium carbonate were obtained from Sigma-Aldrich (St. Louis, MO, USA). Sodium borate was purchased from Thermo Fisher Scientific (Waltham, MA, USA). Glucose, fructose, sucrose, β-carotene, cryptoxanthin and phytofluene were obtained from Sigma-Aldrich (St. Louis, MO, USA). Nanopure water was obtained from NANO pure purification system (Barnstead/Thermolyne, Dubuque, IA). Authentic volatile standards for gas chromatography such as (R)-(+)-limonene, 2-pentylfuran, 6-methylhept-5-en-2-one, ethyl heptanoate, 1-hexanol, dimethyl trisulfide, (Z)-3-hexan-1-ol, ethyl caprylate, 1-octen-3-ol, decanal, benzaldehyde, ethyl butyrate, (*E*)-2-heptenal, (*E,Z*)-2,6-nonadienal, (*E*)-carveol, geranylacetone, β-ionone, benzothiazole, thymol, eucalyptol, 2-methyl-1-butanol, (*E*)-2-hexenal, ethyl hexanoate, octanal, 1-octen-3-ol, nonanal, (*E*)-2-octenal, ethyl (methylthio)acetate, ethyl (methylthio)acetate, 2,6-dimethyl-5-heptenal, hexyl acetate, 2-ethyl-2-hexenal, ethyl hexadecanoate, citronellal, benzyl alcohol, (*E,E*)-2,4-ethylhexadienoate, (*E,E*)-2,4-heptadienal, decanal, 1-octanol, ethyl-3 (methylthio)propionate, (*E*)-2-nonenal, 2,3-butanediol, β-cyclocitral, phenylacetaldehyde, ethyl decanoate, ethyl benzoate, 1-chlorodecane, 1-nonanol, α-terpineol, 1-decanol, α-farnesene, ethyl phenylacetate, α-isomethyl-ionone, β-ionone, β-ionol, 2-phenyl-2-butenal, 1-dodecanol, cinnamaldehyde, 3-phenylpropanol, octanoic acid, nonanoic acid, farnesylacetone, nootkatone (internal standard), n-alkane standards (C5-C24) and SPME fibers were obtained from Sigma-Aldrich (St. Louis, MO, USA). The Extraction efficiency of volatile compounds was improved using sodium chloride (Fisher Scientific, Pittsburg, PA USA).

pH and citric acid activity

pH and citric acid values were measured at room temperature with a pH meter (Mettler Toledo, OH, USA). Three measurements per breeding line were taken and were expressed as the mean ± standard deviation (SD).

Sugar analysis

Sugar analysis was carried out using an HPLC system with a binary pump, autosampler, refractive index detector (Perkin Elmer LC 200 Series, Norwalk, Conn., USA), and Reze x RCM-Monosaccharide Ca+2 (300 × 7.8 mm) column with a guard column Carbo-Ca (4×3 mm ID) (Phenomenex, Inc. Torrance, CA, USA). Briefly, melon samples were blended in a high-speed blender (Oster Blender, Milwaukee, WI, USA) and approximately 5 gm of the pureed sample was mixed with 5 mL nanopure water, homogenized and sonicated. The sample was centrifuged at 10, 000 x g for 15 min. The decant was then mixed with methanol and centrifuged again at 8000 x g for 5 min. The final sample solution was stored at -20 °C until further analysis. The column temperature was maintained at 80 °C and nanopure water, obtained from the NANOPure system (Barnstead/Thermolyne, Dubuque, IA, USA), was used as a mobile phase with a flow rate of 0.6 mL/min. All the samples were prepared in triplicates and the standard curves for glucose, sucrose and fructose were used for the calculation of total sugar content.

Total phenolic content (TPC) Assay

Total phenolic content (TPC) was determined according to our published method with slight modifications.[106] Folin-Ciocalteu (FC) reagent was used to measure the TPC in the melon breeding lines. The FC assay is based on the oxidant reaction between tyrosine/tryptophan,

resulting in the blue color proportional to the protein concentration. The addition of sodium carbonate in the reaction mixture causes the reaction between FC reagent and the phenolic content at pH 10 leading to the dissociation of phenolic proton and formation of phenolate ion.[107] Gallic acid was used as a standard to prepare the calibration curve in the following concentrations, equivalent to 10, 20, 30, 40, 50, 75 and 100 μg. Twenty micro liter of extracted sample in triplicates were added to each well of a Falcon 96-well microplates (Corning Inc., NY, USA); the total volume was adjusted to 200 μL by adding nanopure water in standards and samples. In all the wells, 20 μL of FC reagent was added, and the plate was incubated for 10 min followed by the addition of 50 μL sodium carbonate and further incubated for 20 min. The absorbance was measured at 760 nm using a KC-4 Microplate Reader (BioTek Instruments, Winooski, VT, USA). Results were expressed as gallic acid equivalents (GAE) in μg/g.

DPPH Assay

2,2-Diphenyl-1-Picrylhydrazyl (DPPH) free radical scavenging activity of melon breeding lines was measured as per our published method[108] with a few minor changes. In brief, 40 μL of the extracted sample was added to each well of a Falcon 96-well microplates (Corning Inc., NY, USA) in triplicate and 60 μL of methanol was added to each well. The standards were prepared using ascorbic acid concentrations (equivalent to 10, 20, 30, 40, 50, 75 μg) and the final volume was adjusted to 100 μL with methanol. Following this, 180 μL of DPPH free radical was added to all the wells and the reaction mixture was incubated for 30 min in the dark. The absorbance was measured at 515 nm and the scavenging activity was expressed as ascorbic acid equivalent (AAE) in μg/g.

ABTS radical scavenging activity

2,2′-Azino-Bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay was also analyzed for radical scavenging as per our published method.[21] A fresh reagent was prepared using 7 mmol L-1 aqueous ABTS solution mixed with 2.45 mmol L-1 potassium persulfate aqueous solution, dissolved in nanopure water, and the resultant mixture was stored in the dark for 16 h. The sample extracts (10 μL) were added in triplicates to the wells of Falcon 96-well microplates (Corning Inc., NY, USA). Methanol was used to adjust the total volume to 100 μL. Fresh ascorbic acid standards (equivalent to 5, 10, 15, 20, 25, 30 μg) were added to the wells for measuring the calibration curve and the volume was adjusted to 100 μL with methanol. An aliquot of 180 μL ABTS was then added to all the wells and the absorbance was measured at 734 nm.

Determination and HPLC-PDA analysis of carotenoids

Fresh cantaloupes were cut into two halves, the pulp was removed and cut into small pieces and blended in a high-speed blender at the maximum speed (Oster Blender, 450 W, Milwaukee, WI, USA). The blended puree was mixed with CHCl3:Ace (1:1) and homogenized for 2 min at 10, 000 rpm (850, Homogenizer, Thermo Fisher Scientific, Waltham, MA, USA). The mixture was sonicated for 15 min. The extract was centrifuged for 10 min at 7000 x g. The residue was re-extracted with the above solvents to ensure complete extraction and the resultant supernatants were then pooled and centrifuged at 12, 000 x g for 5 min. The resultant samples were analyzed for carotenoids using a Waters 1525 HPLC system (Milford, MA, USA) equipped with a 2996 DPA detector and 717 Plus autosampler. Mobile phase A and B representing methanol and tert-butyl-methyl ether (TBME) respectively, were used for separation on a C30 column (3 μm, 150 mm x 4.6 mm, YMC Column, Waters Corp) with a flow rate of 0.6 mL/min. A 25 min extraction using a gradient program, 25-75 % B (0-12 min), isocratic (75% B for 8 min), 75-25% B (1 min)

followed by 4 min isocratic at 25% B was used for the separation of carotenoids. Empower-2 software was used for data processing and carotenoids were monitored at 286, 350 and 450 nm.

HS-SPME-GC-MS analysis of volatile compounds

Fresh cantaloupes were cut into four pieces and pulp was separated from the rinds. The pulp was cut into small pieces and blended in a high-speed blender (Oster Blender, Milwaukee, WI, USA) for 1 min. The blended puree from each fruit was weighed in a 20 mL headspace vial. The samples were prepared in triplicates and 1 mL NaCl (30%, w/v) and 5 μL nootkatone as internal standard (0.025%, v/v) were added in each vial. The samples were analyzed using a TriPlus RSH autosampler (Austin, TX, USA). A 50/30 μm carboxen/polydimethylsiloxane/divinylbenzene (CAR/PDMS/DVB) fiber was used for extraction of volatile compounds using HS-SPME. A 30 m x 0.25 mm id with 0.25 μm film thickness Restek Rtx-Wax column was used for analysis (Restek Corp., Bellefonte, PA, USA). A constant agitation at 10 sec every 2 min was employed; the extraction and desorption using SPME fibers were carried out for 30 min and 2 min respectively, at 80 °C. SPME fiber was then injected into the GC injector at 225 °C. Helium was used as a carrier gas with a constant flow rate of 1 mL/min in splitless mode. Thermo Finnegan gas chromatogram coupled with Dual-Stage Quadrupole (DSQII) was used for volatile analysis (Thermo Fisher Scientific, Inc, San Jose, CA, USA; Thermo Fisher, Austin, TX, USA). The initial oven temperature was maintained at 40 °C for 2 min and then increased to 210 °C at a rate of 5 °C/min. The total run time was 37 min. The mass selective detector (MSD) was operated in EI (electronic ionization) mode (70 eV), in a scan mode from 30–300 amu at a rate of 11.5 scans/sec. Ion source and mass transfer line temperature was maintained at 285 °C and 280 °C respectively. An additional positive ionization step was carried out with

methane as a reagent gas at a flow rate of 1 mL/min. The mass transfer line and ion source temperature were 230 °C and 180 °C respectively. The data was processed and recorded using Xcalibur software (v. 2.0.7, Thermo-Fisher Scientific, San Jose, CA, USA).

Statistical analysis

Principal component analysis (PCA) of VOCs was performed using mean data with the help of SIMCA 16.0.2 statistical software (Umetrics Inc., San Jose, CA, USA). All the data were normalized using log transformation to have a normal distribution. The differences in means were calculated using one-way analysis of variance with the XLSTAT software (version 2018.5.52280, Addinsoft, NY, USA). The significance level was fixed at $P < 0.05$ and all the results were represented as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Analysis of physiochemical properties

pH and citric acid values of all the breeding lines from 2018 and 2019 are shown in Table 5. There were no significant differences between the pH and citric acid values in the lines from 2018; The pH ranged from 4.26 ± 0.0 – 4.75 ± 0.03 and citric acid values ranged from 5.31 ± 0.17 g/L – 6.85 ± 1.16 g/L.

Among 2019 lines, the lowest pH was recorded in BL 3-43-1 (5.82 ± 0.22) and the highest pH in BL 2-27-1 (8.0 ± 0.05). Therefore, the citric acid values were low in BL 2-27-1 with 1.45 ± 0.35 g/L and high in BL 3-43-1 with 3.32 ± 0.36 g/L (Table 5). These values are in accordance with the previously published values as reported by[98, 109] for both pH and citric acidity. Most *Cucumis melo* L. cultivars, even with mature fruits, have low organic acid contents which are in contrast to other

fruits such as strawberries, pineapples or apricots that show high acidic content in the mature stage.[98]

Table 5. pH and citric acid values for 2018 and 2019 breeding lines. All the values are expressed as mean ± SE. Means with the same letter indicate no significant difference (p <0.05)

Year	BL	pH	Citric acid (g/L)
2018	BL-96	4.75 ± 0.03a	5.38 ± 0.33a
	BL-53	4.50 ± 0.01ab	6.85 ± 1.16a
	BL-81	4.62 ± 0.03a	5.58 ± 0.89a
	BL-51 X BL-37	4.53 ± 0.03ab	5.31 ± 0.17a
	M26	4.26 ± 0.02b	5.98 ± 0.42a
	BL-136	4.71 ± 0.0a	6.78 ± 0.02a
2019	1-24-1	7.66 ± 0.16a	3.0 ± 0.39a
	1-26-1	6.75 ± 0.18a	2.82 ± 0.39a
	1-40	7.15 ± 0.32a	2.57 ± 0.18a
	2-27-1	8 ± 0.05a	1.45 ± 0.35a
	2-42-1	6.40 ± 0.35a	2.0 ± 0.36a
	2-44-1	6.46 ± 0.07a	2.31 ± 0.42a
	3-14	7.13 ± 0.46a	2.47 ± 0.21a
	3-21-1	7.78 ± 0.15a	2.56 ± 0.45a
	3-22	6.62 ±. 0.47a	2.73 ± 0.19a
	3-28-1	7.12 ± 0.20a	2.63 ± 0.44a
	3-29-1	6.12 ± 0.32a	2.98 ± 0.39a
	3-31-1	6.35 ± 0.35a	3.14 ± 0.39a
	3-34-1	5.98 ± 0.30a	3.22 ± 0.38a
	3-37-1	6.01 ± 0.24a	3.25 ± 0.37a
	3-43-1	5.82 ± 0.22a	3.32 ± 0.36a

Sweetness is the most important consumer preferred quality attribute of melon coupled with its flavor. The levels of glucose, fructose and sucrose were measured in duplicates for each melon fruit. The soluble solid content (SSC) in terms of Brix° was also measured for each fruit. Breeding line 53 had the highest total sugar content (26.3 ± 2.35 mg/g FW) followed by M26 (23.18 ± 1.22 mg/g FW) for 2018 melon samples (Table 6). Similarly, among the 2019 samples, BL 3-43-1 had

the highest total sugar content (54.66 ± 5.4 mg/g FW) (Table 6). A high consumer preference can therefore be attributed to these lines based on their low pH and high acidic contents (Table 5) along with high sweetness levels (Table 6). These levels were previously reported in other studies.[98, 114]

Table 6. Sucrose, glucose and fructose concentrations in 2018 and 2019 breeding lines. All the values are expressed as mean ± SE. The concentrations are in (mg/g FW)

Year	BL	Sucrose	Glucose	Fructose	Total Sugars
2018	BL-96	9.62 ± 0.69	9.25 ± 1.50	–	18.87 ± 0.15
	BL-53	8.56 ± 0.14	12.93 ± 0.03	4.80 ± 0.19	26.30 ± 2.35
	BL-81	9.43 ± 0.46	6.53 ± 0.76	2.00 ± 0.75	17.95 ± 2.16
	BL-51 X BL-37	7.41 ± 1.20	9.73 ± 2.04	4.51 ± 0.56	21.66 ± 1.51
	M26	9.84 ± 0.35	7.74 ± 0.27	5.60 ± 0.83	23.18 ± 1.22
	BL-136	7.87 ± 0.48	7.94 ± 0.22	4.18 ± 0.26	19.99 ± 1.24
2019	1-24-1	1.91 ± 0.17	2.76 ± 0.05	1.23 ± 0.04	5.9 ± 0.45
	1-26-1	2.17 ± 0.03	3.17 ± 0.01	1.46 ± 0.01	6.79 ± 0.5
	1-40	1.22 ± 0.39	2.85 ± 0.29	1.14 ± 0.13	5.2 ± 0.56
	2-27-1	2.24 ± 0.11	2.86 ± 0.03	1.37 ± 0.03	6.46 ± 0.44
	2-42-1	0.72 ± 0.03	3.37 ± 0.01	1.67 ± 0.07	5.75 ± 0.78
	2-44-1	2.14 ± 0.05	4.06 ± 0.03	2.13 ± 0.04	8.32 ± 0.65
	3-14	1 ± 0.25	2.53 ± 0.11	1.15 ± 0.1	4.68 ± 0.49
	3-21-1	1.75 ± 0.05	2.44 ± 0.02	1.16 ± 0.03	5.34 ± 0.37
	3-22	1.09 ± 0.2	3.89 ± 0.31	2.37 ± 0.35	7.34 ± 0.81
	3-28-1	2.26 ± 0.03	3.26 ± 0.01	1.9 ± 0.03	7.42 ± 0.41
	3-29-1	9.8 ± 0.14	11.36 ± 0.21	14.01 ± 0.12	35.17 ± 1.23
	3-31-1	11.18 ± 0.71	14.62 ± 0.94	15.11 ± 0.96	40.9 ± 1.24
	3-34-1	6.17 ± 0.11	8.8 ± 0.05	8.03 ± 0.55	22.99 ± 0.78
	3-37-1	9.5 ± 0.04	9.85 ± 0.25	11 ± 0.43	30.34 ± 0.46
	3-43-1	10.39 ± 0.37	15.72 ± 1.13	28.56 ± 0.55	54.66 ± 5.4

Total phenolics and antioxidant activities

The total phenolic content (TPC) of the breeding lines grown in 2018 and 2019 are shown in Figure 4. Folin–Ciocalteu (FC) reagent was used to determine the TPC at absorbance 760 nm. Significant differences were observed in the TPC among the lines. The highest TPC was observed in BL-53 (93.07 ± 1.86 µg/g) followed by BL-136 (91.86 ± 8.42 µg/g) in 2018 (Fig. 4A). The

lowest TPC was found in BL-81 (51.49 ± 10.63 µg/g). These values were in the similar range (101.90 ± 14.99 µg/g) reported in a previous study.[112] However, higher TPC (218.78 ± 13.09 µg/g) was reported in the commercial cantaloupe varieties in another study.[20]

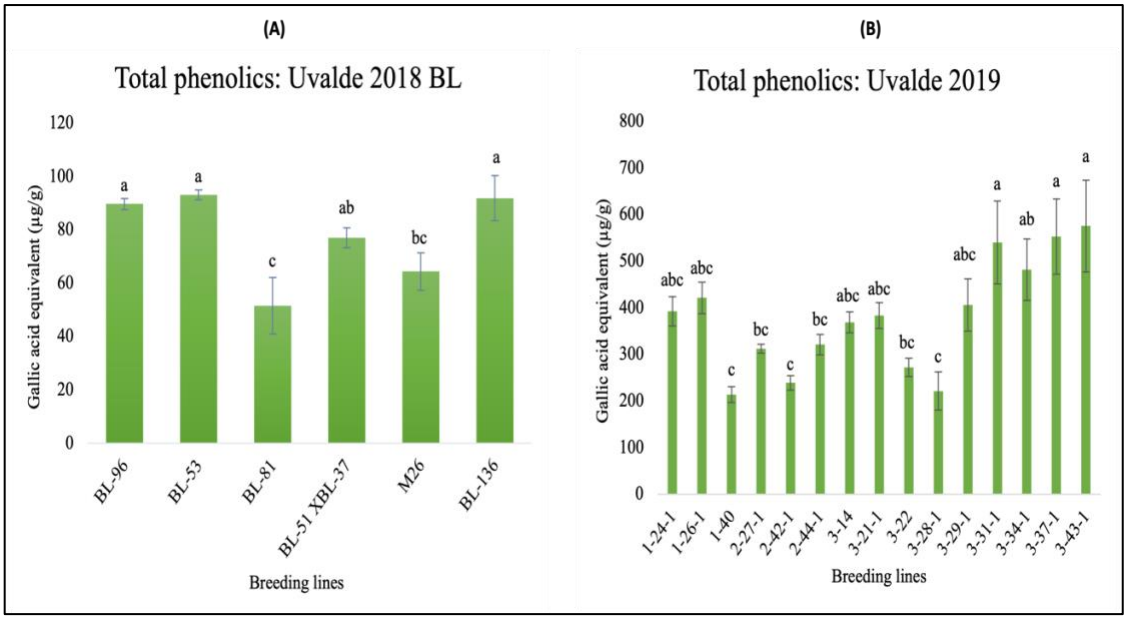

Figure 4. Total phenolic contents (TPC) of Uvalde breeding lines in 2018 and 2019. Fig. (4A) represents 2018 BL and Fig. (4B) represents 2019 BL. Data are represented as mean ± SE. Means with the same letter indicate no significant difference (p <0.05)

Among 2019 samples (Figure 4B), the highest TPC was found in BL 3-43-1 (575.36 ± 98.59 µg/g) and the lowest in BL 1-40 (213.25 ± 16.78 µg/g). The total phenolic content of a fruit, in general, changes due to the activity of polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PLA).[113] It also varies greatly with environmental and genetic conditions,[114] which might explain the variation in the total phenolic content among the lines in both the years.

Determination of total phenolic content and antioxidant activities in fruits and vegetables has gained momentum in recent years.[110] This can be attributed to the fact that phenolic

compounds have high antioxidant activity, free radical scavenging capacity, reducing capacity for reactive oxygen species and are universally present in plants.[110, 111]

The DPPH activity from 2018 lines was high in BL-53 (81.32 ± 4.44 µg/g) and low in BL-96 (37.93 ± 3.12 µg/g). From 2019 lines, the highest activity was in BL 3-31-1 (386.62 ± 44.20 µg/g) and the lowest by 3-22 (52.27 ± 5.33 µg/g). Breeding lines 1-24-1, 1-26-1, 3-29-1, 3-31-1, 3-34-1, 3-37-1 and 3-43-1 had comparatively higher DPPH activity than the other lines, as displayed in Figure 5.

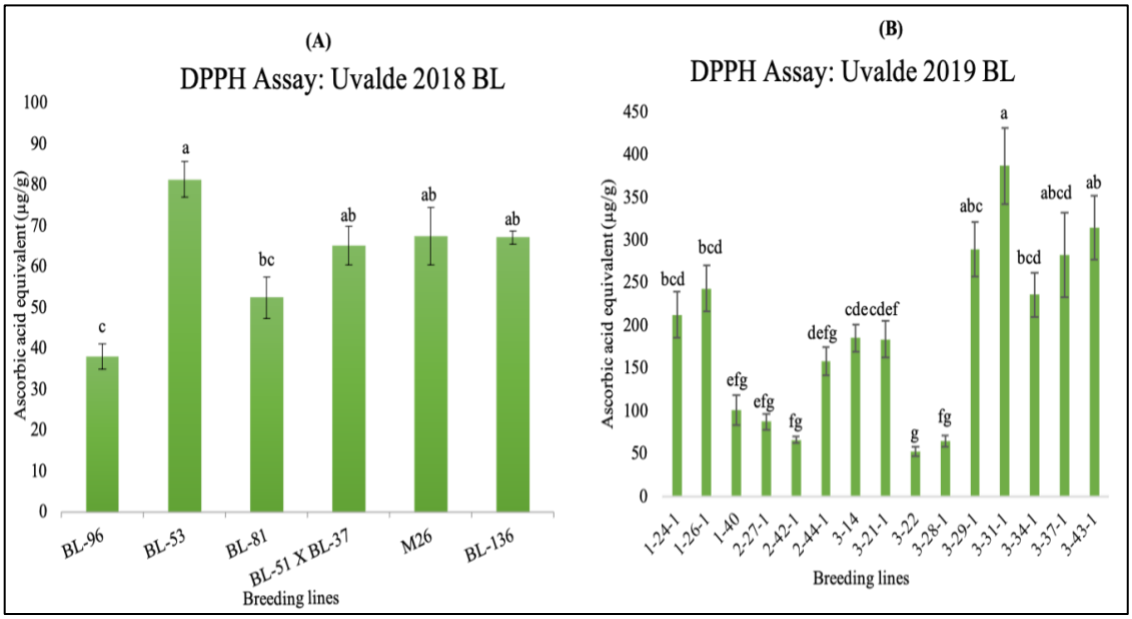

Figure 5. DPPH assay of Uvalde breeding lines in 2018 and 2019. Fig. (5A) represents 2018 BL and Fig. (5B) represents 2019 BL. Data are represented as mean ± SE. Means with the same letter indicate no significant difference (p <0.05)

Results of ABTS activity are as shown in Figure 6. In the year 2018, the lines showed a high scavenging activity in BL-53 (342.78 ± 53.68 µg/g) and the lowest activity in M26 (158.89 ± 10.72 µg/g) (Figure 6A). In the year 2019, BL 3-31-1 (643.12 ± 38.55 µg/g) showed high activity followed by BL 1-26-1 (619.24 ± 39.84 µg/g). The lines that showed higher ABTS activities were

BL 1-24-1, 1-26-1, 3-29-1, 3-31-1, 3-37-1 and 3-43-1. The lowest ABTS activity was found in BL 3-28-1 (62.87 ± 10.20 µg/g) (Figure 6).

Antioxidants are compounds that can inhibit or delay lipid oxidation by preventing the onset of oxidative chain reactions thereby repairing the damaged cells of the body. The mechanisms by which they can act as a defense system range from one or more of the following: singlet oxygen quenching, free radical-scavenging activity and pro-oxidation of metals. It has been previously observed that fruits and vegetables can help fight the body against reactive oxygen species (ROS) due to the activity of their phytonutrients. Free radical scavenging activity can affect the way phytonutrients behave i.e synergistically or antagonistically, which in turn can affect the health and functional properties of food. Consumption of natural antioxidants is therefore reported to have various health benefits.[19, 115-117] In the present study, free radical scavenging activities were determined by performing DPPH and ABTS assays. There is usually a variation observed between the two assays due to their reaction mechanisms; DPPH is based on hydrogen atom transfer whereas ABTS is based on single electron transfer.[21]

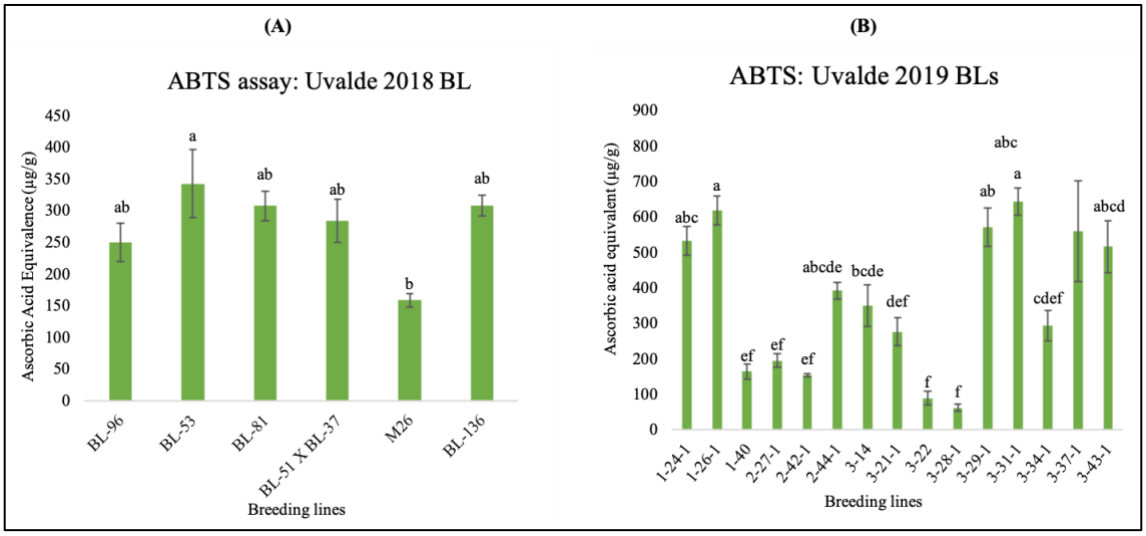

Figure 6. ABTS assay of Uvalde BL in the years 2018 and 2019. Fig. (6A) represents BL in the year 2018 and Fig. (6B) represents BL in the year 2019. Data are represented as mean ± SE. Means with the same letter indicate no significant difference (p <0.05)

The differences in DPPH and ABTS activities can possibly be contributed to the different types of antioxidants present in the fruit; DPPH is scavenged by hydrophobic antioxidants and ABTS is scavenged by both hydrophobic as well as hydrophilic antioxidants. A wide range of pH can be applied across ABTS radical cations (ABTS•+), which also shows a high potency towards most antioxidants and is soluble in both organic and aqueous solvents. On the other hand, the reactivity between DPPH• and antioxidants depend upon the structure of antioxidants making it more complex and comparatively slower than its reactivity with ABTS•+.[20, 118 ,119] Similarly, the differences in the antioxidant activities between the two years could be due to the fact that no overlapping breeding lines were grown in both years. The reason for variability cannot be attributed to one single factor although there have been various studies that show how different varieties, genotypes and cultivars can have a variation on antioxidant activities of foods. Further, it can also be due to environmental effects such as light or temperature.[133, 134, 135]

Analysis of carotenoids

Apocarotenoid, the compounds that are responsible for the aroma of fruits and vegetables, is formed due to the oxidative cleavage of carotenoids.[120] In addition, carotenoids are also reported to have various health-beneficial properties such as protection against cancer, cardiovascular diseases and cataract.[121]

In the present study, carotenoids such as β-carotene and trace amounts of phytoene and phytofluene and ζ-carotene were detected in the 2018 and 2019 breeding lines. In 2018 lines, BL-136 showed the highest β-carotene content (16.55 ± 0.11 µg/g) followed by BL-96 (15.26 ± 1.93 µg/g) (Table 7). β-carotene was not detected in BL M26 and BL-53. Phytofluene and ζ-carotene were detected among all the lines except in M26 and BL-53. The highest phytofluene content was

detected in BL-96 followed by BL-136 with 0.08 ± 0.01 µg/g (Table 7). BL-51 X BL-37 (0.78 ± 0.12 µg/g) had high ζ-carotene content followed by BL-96 (0.61 ± 0.05 µg/g). These values are consistent with the previously reported values for ζ-carotene, phytofluene and β-carotene.[122, 123, 132]

Table 7. β-carotene, Phytofluene and ζ-carotene contents (µg/g) in 2018 and 2019 breeding lines. Values are expressed as mean ± SE. Means with the same letter indicate no significant difference (p <0.05). ND-Not Detected

Year	BL	β-carotene (µg/g)	Phytofluene (µg/g)	ζ-carotene (µg/g)
2018	BL-96	15.26 ± 1.93a	0.08 ± 0.01a	0.61 ± 0.05a
	BL-53	NA	ND	ND
	BL-81	4.13 ± 0.15b	0.04 ± 0.01a	0.24 ± 0.11a
	BL-51 X BL-37	8.11 ± 1.71b	0.07 ± 0.00a	0.78 ± 0.12a
	M26	ND	ND	ND
	BL-136	16.55 ± 0.11a	0.08 ± 0.01a	0.58 ± 0.04a
2019	1-24-1	29.4 ± 5.43ab	0.16 ± 0.01a	1.80 ± 0.31ab
	1-26-1	21.67 ±. 0.74abcd	0.15 ±. 0.01a	2.14 ±. 0.08ab
	1-40	18.59 ± 2.34cd	0.17 ± 0.02a	2.49 ± 0.33ab
	2-27-1	29.55 ± 3.77abc	0.17 ± 0.01a	2.48 ± 0.28ab
	2-42-1	24.97 ± 3.43abcd	0.20 ± 0.03a	3.08 ± 0.62a
	2-44-1	30.52 ± 2.88ab	0.15 ± 0.01a	1.82 ± 0.12ab
	3-14	17.16 ± 1.15d	0.34 ± 0.19a	1.58 ± 0.17b
	3-21-1	18.90 ± 0.48bcd	0.12 ± 0.00a	1.51 ± 0.06b
	3-22	32.63 ± 1.87a	0.19 ± 0.01a	2.07 ± 0.07ab
	3-28-1	24.83 ± 0.87abcd	0.17 ± 0.01a	1.34 ± 0.12b
	3-29-1	ND	ND	ND
	3-31-1	ND	ND	ND
	3-34-1	ND	ND	ND
	3-37-1	ND	ND	ND
	3-43-1	ND	ND	ND

In 2019 lines, β-carotene content ranged from 17.16 ± 1.15 µg/g – 32.63 ± 1.87 µg/g, with the highest in BL 3-22 (32.63 ± 1.87 µg/g) followed by BL 2-44-1 (30.52 ± 2.88 µg/g) and BL 3-14 had the lowest content (Table 7). A high phytofluene content was found in the line 3-14 (0.34 ± 0.19 µg/g) followed by BL 2-42-1 (0.20 ± 0.03 µg/g). The highest ζ-carotene content was observed in BL 2-42-1 (3.08 ± 0.62 µg/g) followed by BL 1-40 (2.49 ± 0.33 µg/g) (Table 7). Due

to lack of samples, β-carotene, phytofluene and ζ-carotene were not determined in BL 3-29-1, 3-31-1, 3-34-1, 3-37-1 and 3-43-1. These carotenoid levels were found to be consistent with our previously published data.[122] There can be a variety of factors that can cause differences in the level of carotenoids amongst different lines and in between the two years, such as maturity time and growing conditions.[124]

Principal component analysis of volatile organic compounds

A total of 72 VOCs were identified from the year 2018 and 109 VOCs from 2019 breeding lines. The major chemical classes of compounds that were identified in both the years were esters, alcohols, aldehydes, ketones and acids. Esters, alcohols and aldehydes are known to be the major contributing classes involved in the melon aroma.[13] Principal component analysis (PCA) was used to observe the correlation between the VOCs and the breeding lines.

In the 2018 breeding lines, PC1 contributed to 28.9% of the total variation and PC2 was responsible for 27.3% of the total variation amongst the BL, as shown in Figure 7. The VOCs were classified into different chemical classes and consisted of 7 esters, 23 aldehydes, 24 alcohols, 5 hydrocarbons, 1 acid, 3 sulfurs and 2 other compounds. As depicted in the figure, the least number of compounds were present in the 1st quadrant (Figure 7B), which doesn't have any BL associated with it (Figure 7A). Most of the ketones and alcohol compounds were predominantly present on the left side of the PC1 axis correlating to breeding lines BL-96, BL-136 and BL-81. Some of the major aroma and flavor inducing compounds correlating with BL-136 and BL-96 are benzaldehyde, citronellal, benzyl alcohol, farnesyl acetone, β-ionone, α-calacorene, (*E*)-2-nonenal and citral.[37, 42, 125-127] On the other hand, the right side of the PC1 axis was mostly affected by the presence of aldehydes (Figure 7B). The positive side of the PC2 axis was affected mainly by the

presence of 2 out of 3 sulfur compounds along with a few aldehydes and alcohols; BL-96 and M26 were found to be correlated with the positive side of the PC2 axis (Figure 7A). Sulfur containing compounds are known for their characteristic aroma in fruits and vegetables and have previously been reported as odorant volatiles in various fruits like pineapples, strawberries and muskmelons.[41, 128] The negative side of the PC2 axis was affected mostly by aldehydes and ketones with the presence of hydrocarbons such as D-limonene and α-calacorene. The remaining 4 breeding lines– BL-96, BL-136, BL-51 X BL-37 and BL-81 were seen to correlate with aforementioned compound classes.

Similarly, in the year 2019, there were 11 esters, 27 aldehydes, 43 alcohols, 10 ketones, 7 hydrocarbons, 4 sulfurs, 4 acids and 3 other compounds present. A total of 109 volatiles were identified. PC1 and PC2 contributed to 16.7% and 15.9% of the total variation, as shown in Fig. 8. It was observed that the positive side of PC1 had the greatest number of volatiles with most of the aldehydes and all of the ketones clustered together. All the 4 acids were also on the right side of the PC1 axis, revealing a great deal of variability in the volatile composition between the BL. On the other hand, most of the alcohols and esters were found to be on the negative side of the PC1 axis correlating with the following breeding lines: 1-26-1, 1-24-1, 2-27-1, 3-29-1, 1-40 and 3-43-1. A major hydrocarbon, D-limonene was also present on the negative side of PC1. The compound D-limonene is known for its antimicrobial and health beneficial properties; it is known to exert antiproliferative effects against various cancer cell types. In addition, limonene is also classified as a generally recognized as safe (GRAS) compound in the Code of Federal Regulation for flavoring agent.[47, 68]

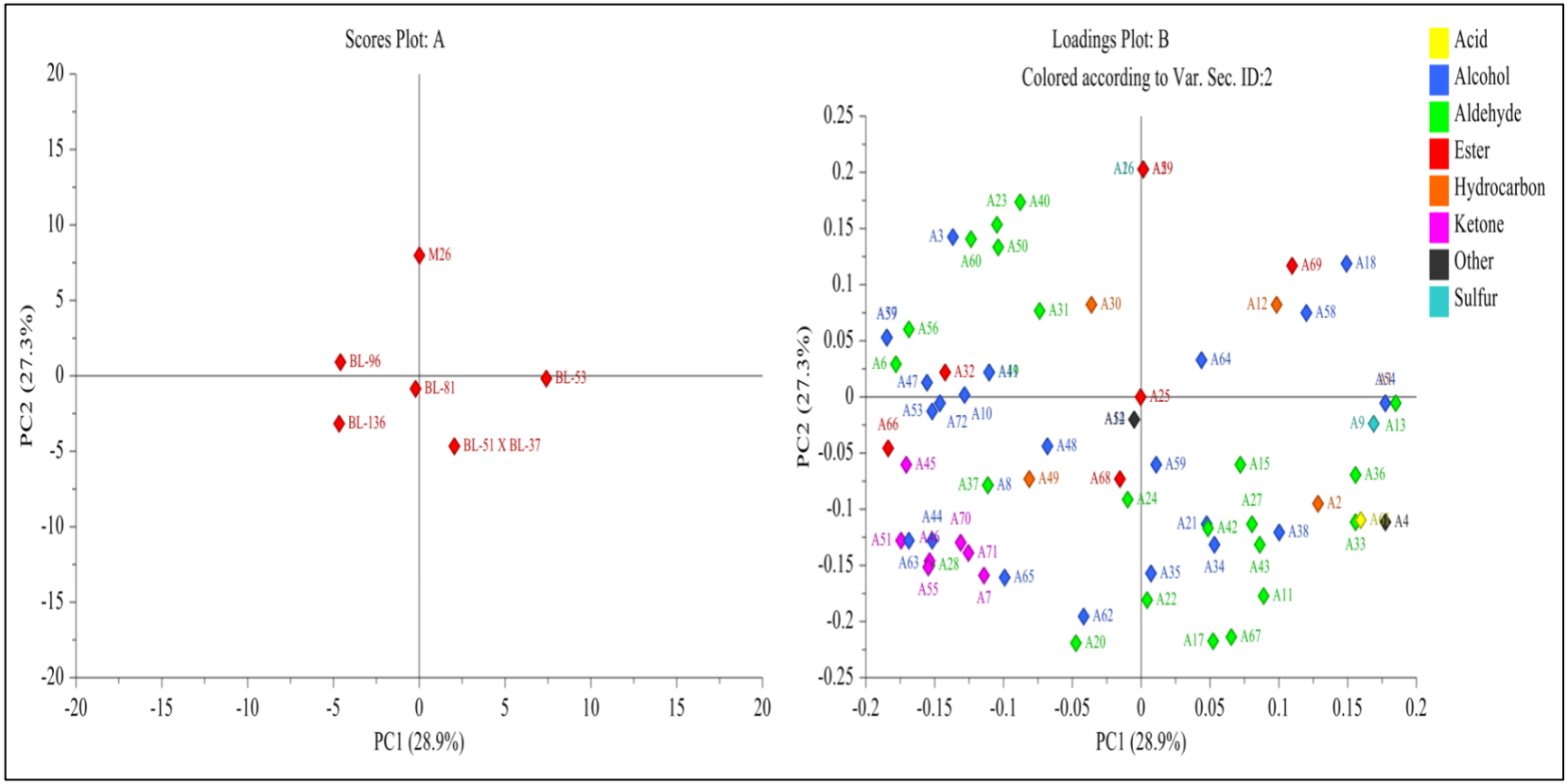

Figure 7. Principal component analysis of Uvalde BL from the year 2018. (A) corresponds to the scores plot showing the BL distribution and (B) corresponds to the loadings plot with the respective volatile codes

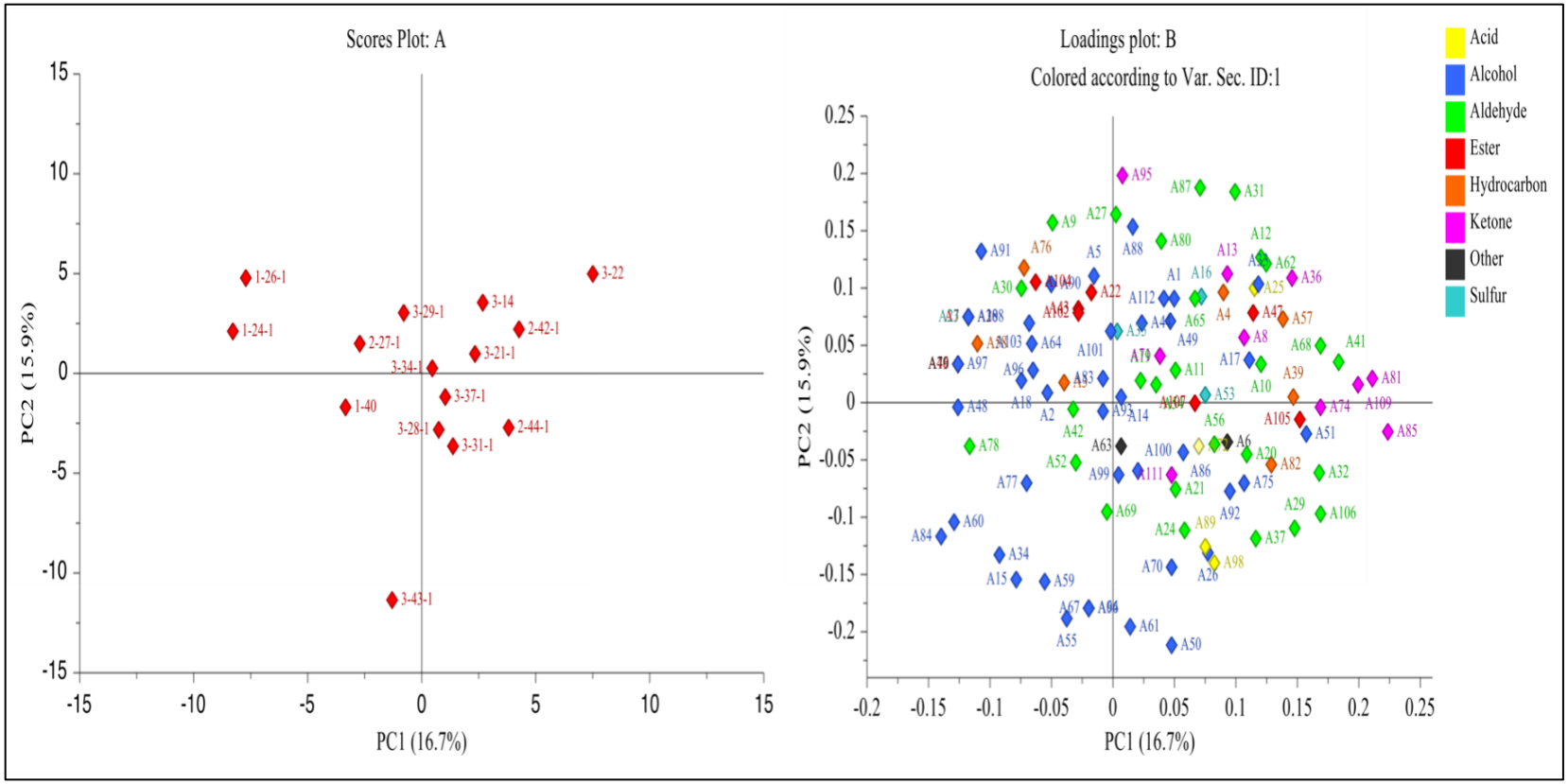

Figure 8. Principal component analysis of Uvalde BL from the year 2019. (A) corresponds to the scores plot showing the BL distribution and (B) corresponds to the loadings plot with the respective volatile codes

After analyzing the PCA results, it was observed that there is an intricate and complex relationship between the volatiles and BL. The breeding lines: 3-43-1 and 3-31-1 were found to have the highest radical scavenging and total phenolic activities. They also showed a high correlation to some of the major aroma compounds such as (*E*,*Z*)-2,6-nonadienal, (*E*,*E*)-2,4-heptadienal, (*E*)-2-nonenal, benzothiazole, geranylacetone, farnesyl acetone, (*Z*)-3-nonenol, 1-octen-3-ol, citral and benzyl alcohol. In addition, the three acidic compounds present i.e hexanoic acid, octanoic acid and nonanoic acid were also found to correlate with these two breeding lines.

CHAPTER V

CONCLUSION

Muskmelon is an important fruit due to its high demand in the market and the presence of various phytonutrients and health-promoting compounds. Our study has gained deep insight into the highly diverse and significant volatile organic compounds (VOCs) present in the different melon breeding lines that possess high organoleptic properties and antimicrobial compounds. These breeding lines were chosen based on durable resistance to multiple diseases, heat tolerance and product quality and were further analyzed for their VOCs. BL-30 and BL-20 showed the highest average VOC content and had the most different VOC classes. For the first time, we identified a new compound, neryl acetone, in muskmelons, which could be further evaluated for their sensory and antibacterial properties. The three main volatiles found in abundance based on their average concentrations are benzaldehyde, geranylacetone, and β-ionone. D-limonene was present in all the breeding lines and has potential efficacy against foodborne pathogens such as *L. monocytogenes*, *S. enterica* and *E. coli*. Moreover, thirty-two compounds identified were previously shown to have antimicrobial properties. Other important lines from this study that could be considered for future projects, based on their health beneficial properties and protective effects against microorganisms, are BL-12, BL-4, BL-7, and BL-9.

In addition to the above lines, we also studied the physicochemical parameters (pH, citric acidity and sugars), carotenoids, VOCs, and antioxidant activities in the breeding lines that were harvested during 2018 and 2019 in Uvalde, Texas. We found that in the year 2018, BL-96 and BL-136 were the most promising, based on their aromatic composition and high carotenoid contents. BL-53 showed high antioxidant activity along with high total sugar content. Similarly, in the 2019 samples, BL 3-43-1 and 3-31-1 were selected due to their high radical scavenging activities.

Additionally, the lines 2-42-1, 2-44-1 and 3-22 had high carotenoid contents. The line 3-43-1 also showed the highest acidic and total sugar contents, making it highly notable with respect to consumer preferences. Some of the major volatile aromatic compounds found in the lines from both years were: geranylacetone, benzyl alcohol, D-limonene, β-ionone, benzaldehyde, (*E*)-2-nonenal and citral that have high antimicrobial properties. Findings from this study could be used to meet consumer preferences by developing high quality melons with high sugar contents, improved antioxidant activities, high carotenoids, and potential VOCs that may serve as biomarkers for improving food safety. We will further explore these breeding lines to screen their peel extracts to determine their efficacy in eradicating foodborne pathogens due to their high antimicrobial content which will enhance the food safety needs.

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