

## Volatile compounds profile of Bromeliaceae flowers

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**Abstract:** Volatile compounds play a vital role in the life cycle of plants, possessing antimicrobial and anti-herbivore activities, and with a significant importance in the food, cosmetic, chemical, and pharmaceutical industry. This study aimed to identify the volatile compounds emitted by flowers of thirteen species belonging to four genera of Bromeliaceae, using headspace solid-phase micro-extraction and detection by gas chromatography-mass spectrometry. A total of 71 volatile compounds belonging to nine chemical groups were identified. The compounds identified represented more than 97 % of the major components in *Aechmea bicolor*, *Ae. bromeliifolia*, *Ae. distichantha*, *Ae. fasciata*, and *Vriesea friburgensis*. In the *Ananas* varieties, over 99 % of the components were identified, and around 90 % in *V. simplex*. *V. friburgensis* presented the largest diversity of volatiles with 31 compounds, while *Alcantarea nahoumii* presented only 14. All three *Ananas* varieties presented the same 28 compounds in relatively similar abundance, which has been confirmed by principal component analysis. Current taxonomy and pollination syndrome studies available can adequately explain the variation in volatile compounds among species. Rev. Biol. Trop. 64 (3): 1101-1116. Epub 2016 September 01.

**Key words:** Bromeliaceae, gas chromatography, headspace, mass spectrometry, principal component analysis, terpenoids, volatile compounds.

Bromeliaceae Juss family belongs to the Poales order, with 58 genus and 3 352 species (Luther, 2012). Bromeliaceae presents a wide diversity of forms and are found in almost every neotropical ecosystem, from sea level in beaches, mangroves, and shoals to altitudes of 4000 m above sea level in the Andes (Benzing, 2000).

Volatile compounds are critical for the plant life cycle, especially for pollination and seed dispersion, which assures plant reproduction and their evolutive success (Pichersky & Gershenzon, 2002; Knudsen & Gershenzon, 2006; Suinyuy, Donaldson, & Johnson, 2013; Aguilar-Rodríguez et al., 2014). Hummingbirds

pollinate most of the bromeliad species, with flowers presenting colorful and attractive bracts and abundant nectar (Benzing, 2000; Kessler & Krömer, 2000; Araujo, Fischer, & Sazima, 2004). Bats are also notable pollinator agents in some species that present scented flowers, of nocturnal anthesis (Sazima, Vogel, & Sazima, 1989; Knudsen & Tollsten, 1995; Sazima, Buzato, & Sazima, 1995; Aguilar-Rodríguez et al., 2014). In addition to ornithophily and chiropterophily, there are also records of pollination of Bromeliaceae by butterflies, bees, and beetles (Benzing, 2000; Kessler & Krömer, 2000; Canela & Sazima, 2005; Siqueira Filho & Machado, 2001; Schmid, Schmid, Zillikens,

& Steiner, 2011). The majority of Bromeliaceae has scentless flowers, except in a few cases such as *Hohenbergia ridleyi* (Baker) Mez. (Siqueira Filho, 1998); in *Tillandsia crocata* (E. Morren) Baker (Gerlach & Schill, 1991); *Canistrum aurantiacum* E. Morren (Siqueira Filho & Machado, 2001); *Puya* sp. (Knudsen, Tollsten, Groth, Bergström, & Raguso, 2004; *Bromelia antiacantha* Bertol. (Canela & Sazima, 2005), and *Tillandsia macropetala* Wawra (Aguilar-Rodríguez et al., 2014). In these species, a delicate and sweet scent is associated with bee pollination. On the other hand, *Encholirium glaziovii* Mez and some *Vriesea* have an unpleasant scent and copious amounts of nectar, attracting bats (Sazima et al., 1989; Sazima et al., 1995). *Bromelia antiacantha* Bertol. flowers have a strong sweet scent which becomes lighter throughout the day (Canela & Sazima, 2005). *Tillandsia macropetala* Wawra flowers are pollinated by bats and present faintly sweet odor in the early hours of the night, when the nectar volume is the highest (Aguilar-Rodríguez et al., 2014).

These compounds may possess antimicrobial and antiherbivore activity, also repelling microorganisms and animals or attracting natural predators, protecting the plant through tritrophic interactions (Hammer, Carson, & Riley, 2003; Arab & Bento, 2006; Lucas-Barbosa, Loon, & Dicke, 2011), which also suggests their involvement in the protection of the reproductive parts of plants during flowering (Kessler, Halitschke, & Poveda, 2011; Parra-Garcés, Caroprese-Araque, Arrieta-Prieto, & Stashenko, 2010). On the other hand, like all inheritable characters, chemical compounds that make up scents may also reflect the taxonomic affinities of a species. The characterization of volatile compounds may contribute to taxonomic and phylogenetic studies considering that some volatile compounds may be specific to certain plant groups (see for example Nogueira, Bittrich, Shepherd, Lopes, & Marsaioli, 2001).

Volatile compounds also have significant importance in the food industry, cosmetics, perfumes, chemical and pharmaceutical industries

(Dudareva & Pichersky, 2006; Cheng, 2010; Darjazi, 2011; Paibon et al., 2011), due to the increased preference for natural food additives and other compounds of biological origin (Huang, Lee, & Chou, 2001). These are mainly terpenoids, phenylpropanoids, hydrocarbons, alcohols, aldehydes, ketones, ethers and esters derived from fatty acids, representing approximately 1 % of the known secondary metabolites in plants (Dudareva, Pichersky, & Gershenzon, 2004). In the Bromeliaceae family, volatile compounds were studied in the *Ananas* genus and mostly the fruit volatile compounds. In *Ananas* over 280 compounds were identified, the most abundant being esters, terpenes, ketones and aldehydes (Tokitomo, Steinhaus, Suttner, & Schieberle, 2005; Liu, Wei, Sum, & Zang, 2008; Wei et al., 2011). Aguilar-Rodríguez et al. (2014) identified nine volatile compounds (three fatty acid derivatives and six terpenoids) in *T. macropetala*, and correlated their presence to bat-pollination during a study of floral and reproductive biology.

This study aims to identify volatile compounds from flowers of thirteen ornamental species belonging to four genera of Bromeliaceae (using headspace solid-phase micro-extraction with detection by gas chromatography-mass spectrometry), and to bring a new contribution revealing the potential that these plants have in the industry, in the synthesis of natural products, as well as in future studies of ecological processes involving plant-animal interactions, and taxonomy studies from the principal components analysis.

## MATERIALS AND METHODS

**Plant material:** Entire flowers were obtained from plants of 13 species belonging to four Bromeliaceae genera: *Aechmea bicolor* L. B. Sm. (ESA 120990), *Ae. bromeliifolia* Baker ex Benth. & Hook. f. (ESA 121275), *Ae. distichantha* Lem. (ESA 121275), *Ae. fasciata* Baker (ESA 120987), *Ae. nudicaulis* Griseb. (ESA 120991), *Ananas macrodotes* E. Morren (ESA 121286), *An. comosus* (L.) Merr. var. *bracteatus* (Lindl.) Coppens & F. Leal

(ESA 121284), *An. comosus* (L.) Merr. var. *erectifolius* (L.B.Sm.) Coppens & F. Leal (ESA 121285), *Alcantarea nahoumii* (Leme) J.R. Grant (ESA 120986), *Vriesea friburgensis* Mez (ESA 121282), *V. michaelii* W. Weber (ESA 121280), *V. paraibica* Wawra (ESA 121276) and *V. simplex* Beer (ESA 120989), grown in a greenhouse, with flowers collected between the months of August 2011 and February 2012. All species presented anthesis between 6:30 and 8 am. Flowers were collected at anthesis (8 am) from the middle part of inflorescences, in three replicates, each flower from a different plant. A representative plant of each species was deposited at the Escola Superior de Agricultura "Luiz de Queiroz" (ESALQ) University of São Paulo (USP) herbarium.

**Solid-phase microextraction:** This technique presents a low cost of execution, good repeatability, quickness, and is solvent-free. Collected flowers were immediately placed in 20 mL capped vials and allowed to equilibrate for 20 min at 37 for 20 °C. Volatile compounds were collected from the headspace of each sample by solid-phase microextraction (SPME), using Supelco SPME fibers coated with divinylbenzene-polydimethylsiloxane (DVB/PDMS, 65 µm) during 20 min at 37 °C. The fiber was then withdrawn into the needle and transferred for injection in the GC-MS system with splitless injection mode at 240 °C (Almeida, Gonçalves, Galego, Miguel, & Costa, 2006).

**Gas chromatography and mass spectrometry:** GC-MS analyses were conducted according to Custódio, Serra, Nogueira, Gonçalves, and Romano (2006) with modifications. The analyses of volatile compounds were performed on a gas chromatograph GC 2 010 (Shimadzu Corp., Kyoto, Japan) coupled to a mass spectrometer QP 2 010 Plus (Shimadzu Corp., Kyoto, Japan). Samples were separated using a capillary column (RTX-5MS 30 m x 0.25 mm x 0.25 µm). The temperature program started at 40 °C for 2 min, increasing at 4 °C per min to 130 °C, remaining at 130 °C for

1 min, increasing at 7 °C per min to 230 °C, remaining at 230 °C for 4 min, totaling 44 min of analysis. Helium was used as the carrier gas at linear velocity of 36.1 cm/s. The interface was maintained at 280 °C and the detector operated in the scanning mode (m/z 45-450). Data integration was performed using the Lab-Solutions-GCMS Ver. 2.5 software (Shimadzu Corp., Kyoto, Japan).

**Volatile identification and semi-quantification:** The volatile compounds were identified by Wiley 138 and FFNSC libraries. The relative abundance of the compounds was calculated based on the MS results.

The data were subjected to the multivariate principal components analysis using Statistica (Statsoft, 2004).

## RESULTS

HS-SPME/GC-MS analysis of the volatile profile emitted by flowers of 13 species belonging to four genera of Bromeliaceae was performed. A total of 80 compounds were extracted and 71 compounds were identified. Nine chemical groups were found in the identified compounds: alcohols, terpenoids, aldehydes, esters, ketones, ethers, furans, oxides, and styrene (Table 1). The compounds identified represented over 97 % of the major components of this fraction. In *Ae. bicolor*, *Ae. bromeliifolia*, *Ae. distichantha*, *Ae. fasciata*, *V. friburgensis* and the three varieties of *Ananas*, over 99.45 % of the compounds were identified, while in *V. simplex* 90.69 % of the compounds were identified (Table 1, and supplementary material).

*V. friburgensis* showed the highest diversity of volatile compounds, with 31 chemicals, while *Al. nahoumii* showed only 14 compounds. The three varieties of *Ananas* contained the same 28 compounds in relatively similar abundance, as verified by principal component analysis (Fig. 1).

The group of terpenes showed the greatest number of compounds and the highest percentage in the fraction analyzed for most species,

TABLE 1  
Percent area of volatile compounds and retention time in a mass spectrum in 13 species  
of Bromeliaceae by HS-SPME/GC-MS

N	Volatile compounds by chemical class	$t_R$ (min) <sup>a</sup>	Bromeliad species (Peak area %)													Aroma descriptor	
			BIC	BRO	DIS	FAS	NUD	BRA	ERE	MAC	ALC	FRI	MIC	PAR	SIM		
<i>Alcohols</i>																	
1	(E)-3-Hexenol	7.75	9.28	6.80	15.57	6.11	18.68										moss <sup>a</sup> , fresh <sup>a</sup>
2	(Z)-3-Hexenol	7.80	24.49														grass <sup>a</sup> , green <sup>b</sup> , fruity <sup>b</sup>
3	n-Hexanol	8.22	6.16	29.68	5.97	2.76	7.97	31.54	2.72	21.21						green <sup>a,b,c</sup> , resin <sup>a</sup> , flower <sup>a</sup> , green <sup>b</sup> , musty <sup>b</sup> , nut <sup>b</sup> , grass <sup>d</sup>	
4	(E)-Sabinene hydrate	15.57														13.82 balsamic <sup>a</sup> , flower <sup>c</sup> , fruity <sup>d</sup>	
5	Phenethyl alcohol	17.29	5.52														sweet <sup>e</sup> , flower <sup>e</sup>
6	p-Menth-2-en-1-ol	17.64														0.59 herb <sup>a</sup>	
7	(Z)-Non-3-en-1-ol	18.74	9.35	2.68												-	
8	Octanol	19.36	2.89														moss <sup>a</sup> , nut <sup>a</sup> , mushroom <sup>a</sup>
9	1-Terpineol	19.73														1.57 lemon <sup>f</sup> , pine <sup>f</sup> , minty <sup>f</sup>	
10	(-)- $\alpha$ -Trepineol	20.23	1.83	2.01	1.69	1.44	6.16	1.55								sweet <sup>b</sup> , fruity <sup>b</sup> , oil <sup>g,h</sup> , flower <sup>g</sup> , anise <sup>h</sup>	
11	Cedrol	32.42	0.93	0.51												cedar <sup>i</sup> , wood <sup>i</sup>	
<i>Terpenes</i>																	
12	$\alpha$ -Thujene <sup>A</sup>	10.29	4.57	1.17	2.91	3.38	4.42									wood <sup>a</sup> , green <sup>a</sup> , herb <sup>a</sup>	
13	1,3, $\delta$ -p-Menthatriene <sup>A</sup>	10.44	4.13	2.33													turpentine <sup>a</sup>
14	$\alpha$ -Pine <sup>A</sup>	10.49	1.26	6.34	1.22	2.03	1.35	3.00	0.89	9.95						pine <sup>a,t,j</sup> , turpentine <sup>a,j</sup> , wood <sup>g,j</sup> , resin <sup>g</sup> , fruity <sup>j</sup> , sweet <sup>j</sup> , green <sup>j</sup> , citrus <sup>j</sup> , lime <sup>j</sup> , camphor <sup>j</sup>	
15	Camphene <sup>A</sup>	11.07	1.28	0.50													camphor <sup>a,t,j</sup> , sweet <sup>j</sup> , fruity <sup>j</sup> , pine <sup>j</sup> , oil <sup>j</sup> , herb <sup>j</sup> , vanilla <sup>j</sup>
16	$\beta$ -Pine <sup>A</sup>	12.13	1.00	3.07	1.54												fresh <sup>g</sup> , pine <sup>a,f,g,j</sup> , resin <sup>a,j</sup> , turpentine <sup>a,j</sup> , musty <sup>j</sup> , green <sup>j</sup> , sweet <sup>j</sup> , wood <sup>j</sup>
17	o-Cymene <sup>A</sup>	12.52	1.31														-
18	$\beta$ -Myrcene <sup>A</sup>	12.67	17.81	0.72	39.26	2.57	24.43	0.47	1.94	1.78	2.07	0.31	0.46	1.58	green <sup>b</sup> , herb <sup>b</sup> , pine <sup>b</sup> , lemon <sup>f</sup> , grapefruit <sup>f</sup> , musty <sup>f</sup> , spicy <sup>f</sup>		
19	Sabinene <sup>A</sup>	12.97	4.33	5.32	0.58	4.95	8.59									5.53 spice <sup>a,i</sup> , turpentine <sup>a,i</sup> , wood <sup>a,i</sup> , citrus <sup>i</sup> , pine <sup>i</sup>	
20	$\alpha$ -Phellandrene <sup>A</sup>	13.16	1.11	0.98	1.19											0.91 spice <sup>a,j</sup> , turpentine <sup>a</sup> , mint <sup>a,j</sup> , fruity <sup>j</sup> , herb <sup>j</sup> , citrus <sup>j</sup> , lime <sup>j</sup>	
21	$\delta$ -3-Carene <sup>A</sup>	13.33	6.30														lemon <sup>a</sup> , resin <sup>a</sup>



TABLE 1 (Continued)

N	Volatile compounds by chemical class	$t_R$ (min) <sup>a</sup>	Bromeliad species (Peak area %)							Aroma descriptor									
			BIC	BRO	DIS	FAS	NUD	ERE	MAC		ALC	FRI	MIC	PAR	SIM				
22	$\alpha$ -Terpinene <sup>A</sup>	13.70							5.73	11.25	7.55			6.75			5.56	green <sup>b</sup> , citrus <sup>b</sup> , lemon <sup>a</sup>	
23	<i>p</i> -Cymene <sup>A</sup>	13.95							1.39	6.32	5.07			3.83			3.74	solvent <sup>a,j</sup> , gasoline <sup>a,j</sup> , citrus <sup>a,j</sup> , lemon <sup>i</sup> , fruity <sup>j</sup> , sweet <sup>j</sup> , spicy <sup>j</sup>	
24	Sylvestrene <sup>A</sup>	14.03			2.07												-		
25	( <i>E</i> )- $\beta$ -Ocimene <sup>A</sup>	14.04	0.53	3.02	0.56	5.98								0.20	10.23	1.24	0.14	sweet <sup>a,b</sup> , herb <sup>a,b</sup>	
26	Limonene <sup>A</sup>	14.11				1.25												lemon <sup>a</sup> , orange <sup>a</sup> , citrus <sup>a,f,g</sup> , minty <sup>f</sup> , green <sup>g</sup>	
27	$\gamma$ -Terpinene <sup>A</sup>	15.35							8.06	18.43	12.44			10.47			7.85	gasoline <sup>a</sup> , turpentine <sup>a</sup> , sweet <sup>f</sup> citrus <sup>f</sup>	
28	Terpinolene <sup>A</sup>	16.37							2.88	4.69	6.08			3.52			3.40	flower <sup>b</sup> , lemon <sup>b</sup> , fruity <sup>b,f</sup> , green <sup>f</sup> , wood <sup>j</sup> , sweet <sup>j</sup> , pine <sup>j</sup> , anise <sup>j</sup> , plastic <sup>j</sup>	
29	Linalool <sup>A</sup>	16.85										12.94						flower <sup>a,b,g,j</sup> , fruity <sup>f,j</sup> , lavender <sup>a,j,k</sup> , sweet <sup>b,g</sup> , herb <sup>d</sup> , spicy <sup>d</sup> , citrus <sup>j,k</sup> , anise <sup>j</sup> , muscal <sup>b,j</sup> , parsley <sup>j</sup>	
30	4,8-dimethylhona-1,3,7-triene <sup>B</sup>	17.40				4.46						30.69			1.41	2.73	-		
31	Bicyclolemane <sup>C</sup>	25.32							2.34	1.05	1.68			2.80			3.74	-	
32	$\alpha$ -Cubebene <sup>C</sup>	25.76							1.30	0.49	0.79			1.01			1.32	herb <sup>a</sup> , wax <sup>a</sup>	
33	Isolodene <sup>C</sup>	26.59				2.69											-		
34	$\alpha$ -Copaene <sup>C</sup>	26.62				1.41			0.77	0.69	0.86			0.62			1.57	wood <sup>a,j</sup> , spice <sup>a,j</sup> , earthy <sup>j</sup>	
35	$\beta$ -Elemene <sup>C</sup>	27.18	0.40						0.92	0.23	0.58			0.77			1.05	herb <sup>a</sup> , wax <sup>a</sup> , fresh <sup>a</sup>	
36	$\beta$ -Maaliene <sup>C</sup>	27.74							1.97	2.05	2.70			0.12			-		
37	$\alpha$ -Gurjunene <sup>C</sup>	27.80												2.13			2.53	wood <sup>a</sup> , balsamic <sup>a</sup>	
38	( <i>E</i> )-Caryophyllene <sup>C</sup>	28.06				2.46	8.05		1.77	1.33	1.66						-		
39	Caryophyllene <sup>C</sup>	28.08	18.18	10.19					2.69	2.12	2.23			1.85			0.55	2.27	musty <sup>j</sup> , green <sup>j</sup> , spicy <sup>j</sup> , wood <sup>j</sup> , fruity <sup>j</sup> , sweet <sup>j</sup> , terpenine <sup>j</sup>
40	$\gamma$ -Maaliene <sup>C</sup>	28.29							0.78	0.57	0.73			0.68			-		
41	( <i>E</i> )- $\alpha$ -Bergamotene <sup>C</sup>	28.39	4.52	2.20		1.85												wood <sup>a</sup> , warm <sup>a</sup> , tea <sup>a</sup>	
42	$\alpha$ -Maaliene <sup>C</sup>	28.51												1.12			1.42	-	
43	Alloaromadendrene <sup>C</sup>	28.63							5.04	3.99	3.90			4.82			5.46	wood <sup>a</sup>	
44	Cadina-3,5-diene <sup>C</sup>	28.87				4.86			1.00	0.66	0.86			0.90	1.26		-		
45	$\alpha$ -Humulene <sup>C</sup>	28.99	21.19	8.91		9.63												wood <sup>a</sup> , fresh <sup>g</sup>	
46	$\alpha$ -Selinene <sup>C</sup>	29.14	2.69	1.54	1.74													-	
47	Muurola-4(14),5-diene <sup>C</sup>	29.17				8.96												-	
48	Neo-allo-ocimene <sup>A</sup>	29.23		0.60										1.87	0.17	4.43	2.15	-	

TABLE 1 (Continued)

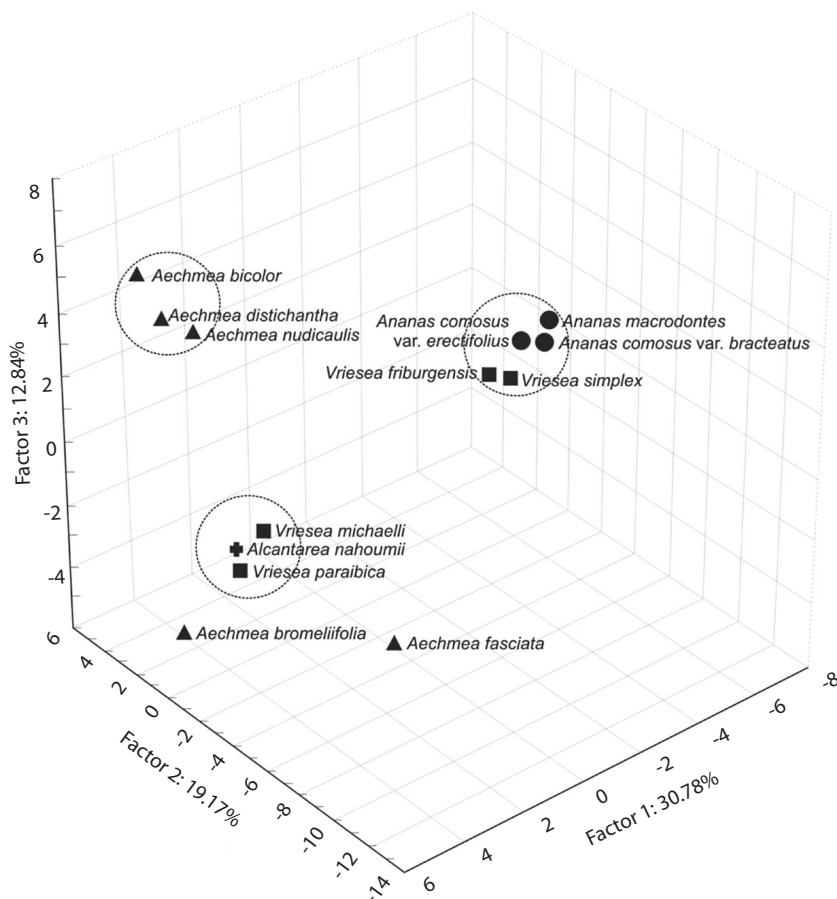
N	Volatile compounds by chemical class	$t_r$ (min) <sup>a</sup>	Bromeliad species (Peak area %)										Aroma descriptor							
			BIC	BRO	DIS	FAS	NUD	BRA	ERE	MAC	ALC	FRI		MIC	PAR	SIM				
49	Cadina-1(6),4-diene <sup>C</sup>	29.48							2.39	1.45	1.93			1.83		1.83	-			
50	$\beta$ -Selimene <sup>C</sup>	29.79	2.30		0.59		0.78										herb <sup>a</sup>			
51	$\delta$ -Elemene <sup>C</sup>	30.01	3.17		0.98												wood <sup>a</sup>			
52	Selina-3,7(11)-diene <sup>C</sup>	30.04					1.50										-			
53	Viridiflorene <sup>C</sup>	30.06							8.50	5.35	5.52						-			
54	$\gamma$ -Elemene <sup>C</sup>	30.19										4.98					6.36	green <sup>a</sup> , wood <sup>a</sup> , oil <sup>a</sup>		
55	$\beta$ -Bisabolene <sup>C</sup>	30.23	2.38		0.70		1.49											balsamic <sup>a</sup>		
56	$\beta$ -Curcumene <sup>C</sup>	30.30	0.75			2.55												-		
57	$\delta$ -Cadinene <sup>C</sup>	30.71				5.00			4.37	2.79	2.33		3.08				4.05	thyme <sup>a</sup> , wood <sup>a</sup> ,		
58	$\alpha$ -Cubenene <sup>C</sup>	30.91							1.62	0.81	0.76		0.88				1.02	gerb <sup>f</sup>		
59	Squalene <sup>B</sup>	31.79										1.28						0.93		
Aldehydes																				
60	<i>n</i> -Hexanal	6.09		3.41								4.22						apple <sup>c</sup> , green <sup>c</sup> , grassy <sup>c</sup>		
61	Phenylacetaldehyde	14.66		8.17										1.32				apple <sup>j</sup> , apricot <sup>j</sup> , cherry <sup>j</sup> , chocolate <sup>j</sup> , grape <sup>j</sup> , honey <sup>j</sup> , hyacinth <sup>j</sup> , lemon <sup>j</sup> , melon <sup>j</sup> , orange <sup>j</sup> , green <sup>j</sup> , nut <sup>j</sup> , fruity <sup>j</sup> , peach <sup>j</sup> , peanut <sup>j</sup> , vegetable <sup>j</sup> , sweet <sup>j</sup> , flower <sup>j</sup> , daisy <sup>j</sup>		
62	<i>n</i> -Nonanal	16.91	1.00	0.61	0.42	5.01	1.18					0.15		0.4				fat <sup>a</sup> , citrus <sup>a</sup> , green <sup>a</sup>		
63	( <i>E</i> )-Non-2-enal	18.94	1.68	0.98	2.77	2.48	0.84							0.93	0.82			metallic <sup>b</sup> , herb <sup>b</sup> , green <sup>a,b</sup> , cucumber <sup>a</sup> , fat <sup>a,l</sup> , soap <sup>i</sup> , clorin <sup>m</sup>		
64	Decanal	20.61	1.62	0.20	0.23	1.19	0.56							0.35	0.51			soap <sup>a</sup> , citrus <sup>a</sup> , fallow <sup>a</sup> , fruity <sup>f</sup> , balsamic <sup>f</sup> , geranium <sup>l</sup>		
Esters																				
65	Lyranion	32.11				0.83	0.52												sweet <sup>i</sup> , orange blossom <sup>i</sup>	
66	Diisobutyl phthalate	36.85	0.55			1.96									0.51				-	
Ketone																				
67	Gauthieric acid	20.29	0.53	1.01			1.03					2.09	1.52	63.13	10.02				-	
Ether																				
68	Eucalyptol	14.02							6.08	5.37	5.16		5.58						5.26	fresh <sup>i</sup> , camphor <sup>i</sup>
Furan																				
69	2-Pentyl-furan	12.66	0.70	0.92								0.54		0.77	0.93					butter <sup>a</sup> , bean <sup>a,e</sup> , green <sup>a,e</sup> , green <sup>a,e</sup> , fruit <sup>e</sup> , vegetables <sup>e</sup> , soil <sup>e</sup> , root incense <sup>e</sup>

TABLE 1 (Continued)

N	Volatile compounds by chemical class	$t_R$ (min) <sup>a</sup>	Bromeliad species (Peak area %)						Aroma descriptor								
			BIC	BRO	DIS	FAS	NUD	BRA		ERE	MAC	ALC	FRI	MIC	PAR	SIM	
70	(Z)-Linalool oxide	16.42										0.99					flower <sup>a</sup> , resin <sup>d</sup> , citrus <sup>d</sup>
71	Styrene	8.94				4.45											balsamic <sup>b,k</sup> , gasoline <sup>a,k</sup> , penetrating <sup>k</sup>
72	Unknown 1	6.82		1.32								0.23		0.22	2.20		-
73	Unknown 2	12.92				4.07	1.43					1.16		2.05			-
74	Unknown 3	12.95		1.32											1.84		-
75	Unknown 4	15.58		1.24	0.42												-
76	Unknown 5	18.83	2.00	13.24	5.00							1.12		2.35	2.89		-
77	Unknown 6	21.94	0.17	0.85	0.44												-
78	Unknown 7	24.76	0.23	0.38	0.12	1.42	0.30					0.27		0.61	0.62		-
79	Unknown 8	28.92				1.26											-
80	Unknown 9	29.87				5.66									0.37		-

BIC = *Aechmea bicolor*; BRO = *Ae. bromeliifolia*; DIS = *Ae. distichantha*; FAS = *Ae. fasciata*; NUD = *Ae. nudicaulis*; ALC = *Alcantarea nahoumii*; BRA = *Ananas comosus* var. *bracteatus*; ERE = *An. comosus* var. *erectifolius*; MAC = *An. macrodontes*; FRI = *Vriesea friburgensis*; MIC = *V. michaelii*; PAR = *V. paraibica*; SIM = *V. simplex*; A = monoterpene; B = homoterpene; C = sesquiterpene; D = triterpene.

a = Acree, & Arm (2004); b = Mahattanatawee et al. (2005); c = Fan, & Quian (2006); d = Phi, Nishiyama, Choi, & Sawamura (2006); e = Zheng, Kim, Kim, Leem, & Lee (2004); f = Qiao et al. (2008); g = Cuevas-Glory et al. (2013); h = Bordiga et al. (2013); i = Brechtill (2007); j = Formisano et al. (2009); k = Lee, Chambers, Chambers, Adhikari, & Yoon (2013); l = Wang, Hossain, Perry, Adams, & Lin (2012); m = Culleré et al. (2013).



**Fig. 1.** Dispersion diagram of scores associated with the first three principal components obtained from the analysis of volatile compounds from flowers of 13 species of Bromeliaceae by HS-SPME/GC-MS.

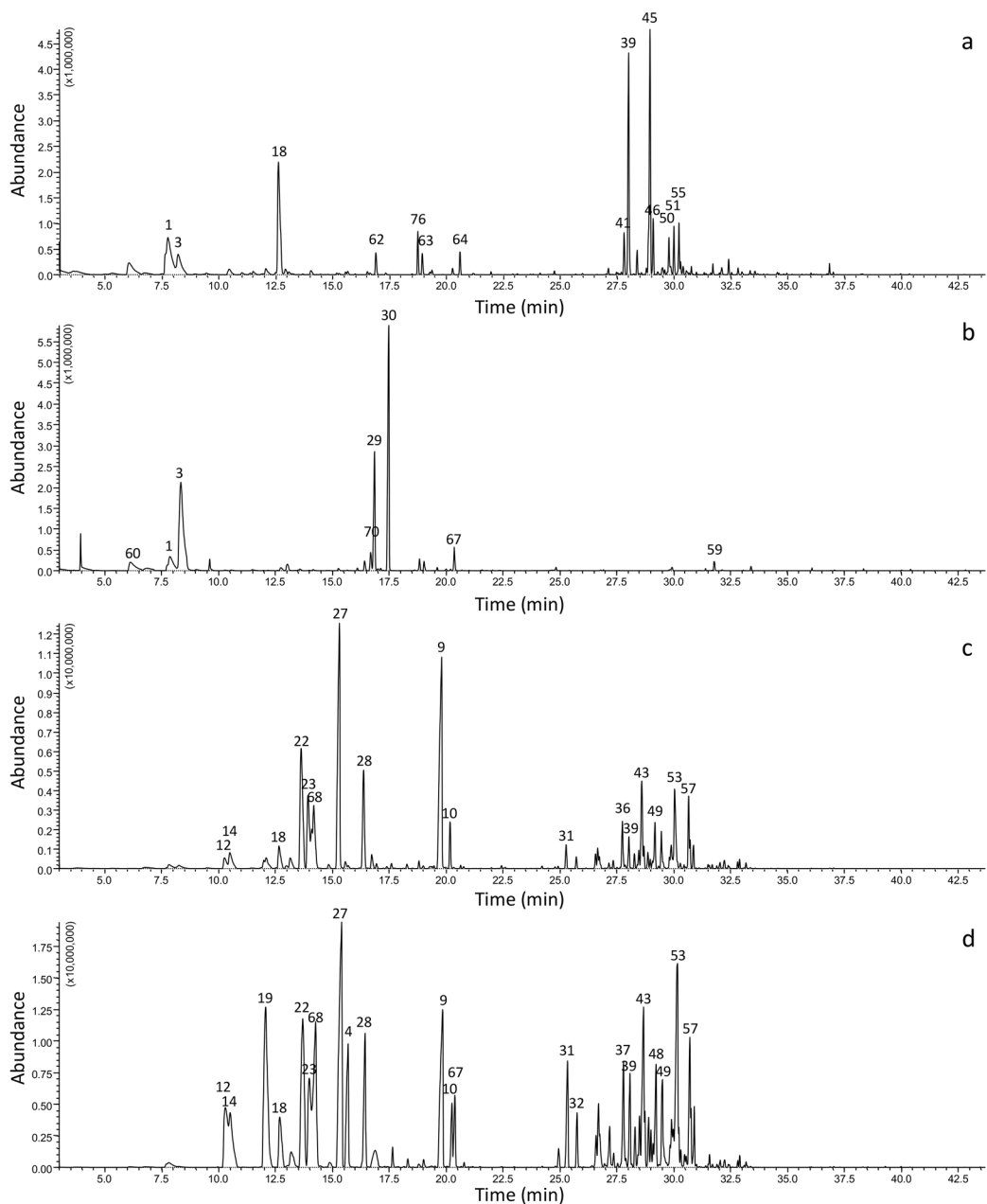
such as *Ae. bicolor* (74.92 %), *Ae. distichantha* (77.54 %) and *V. friburgensis* (71.27 %). Among the *Ananas* varieties, terpenes corresponded to 69.01 %, representing 66.21 % (*A. comosus* var. *bracteatus*), and 70.97 % (*A. comosus* var. *erectifolius*) of the total composition. The compound  $\beta$ -myrcene was observed in all species except *Al. nahoumii*, with abundance ranging from 0.31 to 39.26 % in *V. michaeli*, and *Ae. distichantha*, respectively. Linalool was only observed in *Al. nahoumii*, with approximately 12.94 % of the total compounds found (Table 1, Fig. 2B, and supplementary material).

Eleven compounds belonging to the class of alcohols were identified, with a total

abundance ranging from 6.12 % to 59.69 % in *V. michaeli* and *Ae. bromeliifolia*, respectively. Among them, the most abundant were *n*-hexanol, (*Z*)-hex-3-enol, (*E*)-sabinene hydrate and 1-terpineol (Table 1). *n*-Hexanol was identified in high abundance in most species studied, particularly *Al. nahoumii* (31.54 %), *Ae. bromeliifolia* (29.68 %) and *V. paraibica* (21.21 %).

Five aldehydes were identified among the compounds: *n*-hexanal, phenylacetaldehyde, *n*-nonanal, (*E*)-non-2-enal and decanal, the last three being found in all species of *Aechmea* analyzed. Phenylacetaldehyde was observed in *Ae. bromeliifolia* (8.17 %), and *V. michaeli* (1.32 %).





**Fig. 2.** Chromatographic profile obtained by HS-SPME/GC-MS of volatile compounds in flowers of Bromeliaceae, indicating the major compounds identified: A) *Aechmea bicolor*, B) *Alcantarea nahoumii*, C) *Ananas comosus* var. *erectifolius*, D) *Vriesea friburgensis*.

Gaultheric acid was found in highest abundance in *V. michaelii* (63.13 %), *V. paraibica* (10.02 %), while in the other species the values were below 2 % (Table 1). Eucalyptol, an ether,

was emitted by flowers of the three varieties of the genus *Ananas* (Fig. 2C), *V. friburgensis* (Fig. 2D) and *V. simplex*, with abundance ranging from 5.16 to 6.08 % (Table 1).

TABLE 2

Parameters obtained from the principal components analysis detailing the first five principal components obtained by HS-SPME/GC-MS from volatile compounds emitted by flowers of 13 species of Bromeliaceae

Principal components	Eigenvalue	Total variance %	Accumulated eigenvalue	Accumulated percentage
PC 1: eucalyptol; alloaromadendrene; cadina-1(6),4-diene	25.2458	30.7876	25.2458	30.7876
PC 2: cadina-3,5-diene; unknown 7; muuro-la-4(14),5-diene	15.7234	19.1749	40.9693	49.9625
PC 3: $\alpha$ -humulene; ( <i>E</i> )- $\alpha$ -bergamotene; $\beta$ -bisabolene	10.5336	12.8459	51.5029	62.8084
PC 4	6.3267	7.7155	57.8296	70.5239
PC 5	5.1625	6.2958	62.9922	76.8198

The principal component analysis based on the volatile compounds of Bromeliaceae flowers from 13 species showed the first three components retaining 62.81 % of the initial information (Table 2). This total variance is considered high, with high heterogeneity of the samples' chemical composition.

The correlation of each component and the variables allowed for the evaluation of the discriminatory power of this analysis. For PC1 (30.78 %), the variables that mostly influenced the separation of accessions based on volatile compounds were the presence of eucalyptol, alloaromadendren, and cadina-1(6),4-diene, with correlation values of 0.99, 0.98 and 0.98, respectively. For PC2 (19.17 %), the variables with highest influence were cadina-3,5-diene, unknown 7 and muuro-la-4(14),5-diene, with correlation values of 0.96, 0.93 and 0.93, respectively. For PC3 (12.85 %), the most significant variables were  $\alpha$ -humulene, (*E*)- $\alpha$ -bergamotene and  $\beta$ -bisabolene, with correlations of 0.76, 0.75 and 0.75, respectively.

The dispersion diagram of the scores of the first three main components (Fig. 1) showed three groups, and two isolated species, demonstrating the variability of volatile compounds among these bromeliad species.

## DISCUSSION

This study demonstrated great variability of volatile compounds among the bromeliad species, with 71 compounds identified belonging to eight chemical groups, including

alcohols, terpenoids, aldehydes, esters, ketones, ethers, furans, oxides, and styrene.

The presence of terpenoids in plants is associated with defense against herbivores, pathogenicity, and allelopathy to attract pollinators (Langenheim, 1994). Terpenes are associated with various fragrances, making them widely used to make perfumes and flavorings (Bauer, Garbe, & Surburg, 2001).

The compound  $\beta$ -myrcene has been described in more than 200 plant species as being responsible for green, herb, pine, lemon, grapefruit, musty and spicy scents (Mahattana-tawee, Rouseff, Filomena, & Naim, 2005; Qiao et al., 2008). It is widely used in the cosmetic and pharmaceutical industries, as described by Behr and Johnen (2009). Linalool has been observed in tangerines and is responsible for their taste and aroma (Sawamura, Minhtu, Onishi, Ogawa, & Choi, 2004).

Two other compounds observed in high abundance in most species studied here were:  $\gamma$ -terpinene, which has a citrusy, sweet (Acree & Arn, 2004), gasoline and turpentine scent (Qiao et al., 2008); and  $\alpha$ -humulene, which has a woody (Acree & Arn, 2004) and fresh scent (Cuevas-Glory, Ortiz-Vezquez, Sauri-Duch, & Pino, 2013) and has been shown to have anti-inflammatory properties. It is also found in the essential oil of *Cordia verbenacea* DC (Boraginaceae) (Fernandes et al., 2007).

Aldehydes have strong odors that recall citrus fruits, roses and fresh cut grass, being widely used in perfumery (Bauer et al., 2001). Phenylacetaldehyde is an aromatic compound also found in *Fagopyrum esculentum* Moench

(Polygonaceae) (Janes, Kantar, Kreft, & Prosen, 2009) and several species of flowers (Robert & Meagher, 2002). This compound has long been used to attract various species of moths in traps for biological control (Robert, & Meagher, 2002; Smith, Allen, & Nelson, 1943; Cantelo, & Jacobson, 1979), and has a floral/honey odor (Whetstone, Cadwallader, & Drake, 2005).

High abundance of n-hexanol has also been observed in plum fruits, *Prunus domestica* L. (Rosaceae) (Gomez, Ledbetter, & Hartsell, 1993), *Caralluma europaea* (Guss.) N.E.Br. (Apocynaceae) flowers (Formisano et al., 2009), *Camellia sinensis* (L.) Kuntze (Theaceae) (Han, Zhou, Cui, & Fu, 2006) and essential oil of *Pimenta guatemalensis* (Lundell) Lundell (Chaverri & Ciccio, 2015). Jabalpurwala, Smoot and Rouseff (2009), studying volatile compounds in flowers of different species of *Citrus*, also observed high levels of n-hexanol in *Citrus grandis* (L.) Osbeck (Rutaceae), and correlated the levels with the pollination by bees, which is crucial for reproduction due to self-incompatibility.

Gaultheric acid belongs to the class of ketones and can be found in wines and plant species such as *Gaultheria itoana* Hayata (Ericaceae) (Chen et al., 2009). It is also abundant in the root bark of *Securidaca longepedunculata* Fresen (Polygalaceae), exerting a biocide effect against insects that feed on stored grains (Lognay, Marlier, Seck, & Haubruge, 2000). The emission of volatile compounds with biocide effect can be related to pollination, exerting a repellent effect on some insect species.

Eucalyptol was also identified among the volatile compounds in African cycad (*Encephalartos*) (Zamiaceae) flowers (Suinyuy et al., 2013). Furans and oxides were present in a few species studied, but in low amounts.

Special patterns of scent in flowers can function as the same visual patterns, so differences in intensity and types of volatile compounds emitted, besides serving as guides for insects, help in the search for food rewards. A combination of chemical analyses of floral scents with field observations of the behavior of flower visitors is an effective way to

demonstrate the effect of volatiles in the attraction of pollinators (Dobson, 1994).

The dispersion diagram of the first three main components scores showed *V. michaelli*, *V. paraibica* and *Al. nahoumii* forming the first group. These species are phylogenetically close, belonging to the subfamily Tillandsioideae (Barfuss, Samuel, Till, & Stuessy, 2005; Givnish et al., 2011; Versieux et al., 2012), and *Alcantarea* traditionally being either considered as a subgenus of *Vriesea*, or a genus itself, both belonging to the tribe *Vrieseae* (Grant, 1995). Floral morphology is one of the factors that influence the pollination syndrome (Aguilar-Rodríguez et al., 2014), these species present large flowers with yellow petals, and a tubular corolla. Similarities in composition of the flower volatiles produced among these species can be another factor to consider in establishing the relationship of these species.

*Ae. bicolor*, *Ae. distichantha*, and *Ae. nudicaulis* belong to the Bromelioideae subfamily, with similar flower morphological characteristics. They formed a second group according to their volatile compound composition. Faria, Wend and Brown (2004), studying the cladistic relationships of this genus, showed that *Ae. distichantha* and *Ae. nudicaulis* are very close in clade distribution. In addition, there are reports of common pollinators for these two species (Schmid, Schmid, Zillikens, Harter-Marques, & Steiner, 2010; Srok & Varassin, 2011). There are very limited studies of *Ae. bicolor*, however morphological similarity is observed between its inflorescence and flower morphological characteristics and those of *Ae. nudicaulis*.

The dispersion diagram showed the two other *Aechmea* species studied, *Ae. fasciata* and *Ae. bromeliifolia*, isolated in the principal component analysis of the volatile compounds. This demonstrates the considerable variability of flower volatile compounds among this genus.

The third group included *An. macrodotes*, *An. comosus* var. *erectifolius* and *An. comosus* var. *bracteatus* and two species of *Vriesea* (*V. simplex* and *V. friburgensis*). For *Ananas*, this grouping supports the morphological and

taxonomic closeness within the genus, considering that the same 28 compounds were observed in *Ananas* flowers at similar values.

Considering that the flower volatile compound spectrum can be a plant strategy to attract pollinators, the two species of *Vriesea* may present pollination syndrome similarities with the genus *Ananas*, thus being grouped together in the principal component analysis. Hummingbirds are common pollinators between these two genus, which can explain the proximity of these species in the PCA results. Regarding *Ananas ananassoides*, Stah, Nepi, Galetto, Guimarães, and Machado (2012) observed the presence of two Trochilidae species, *Hylocharis chrysura* and *Thalassidroma glaucopsis*, the latest also observed by Schmid et al. (2011) in *V. friburgensis*.

One of the reports of volatile compounds in bromeliads refers to *T. macropetala*, in which Aguilar-Rodríguez et al. (2014) identified nine volatile compounds, two of them similar to those found in this study, namely nonanal in the species *Aechmea*, *Al. nahoumii* and *V. michaelii*, and limonene in *Ae. nudicaulis*. Those authors pointed out that the pollination syndrome is not necessarily related to a single compound, such as dimethyl disulphide, which although absent in *T. macropetala*, did not prevent the visit of pollinating bats. In this case, of the nine compounds identified by the researchers, six are also present in other species pollinated by bats. In *Weruohia gladioliflora*, also pollinated by bats, Bestmann, Winkle, and Helversen (1997) observed 12 volatile compounds, five of them common to those observed in the species of the present work ( $\alpha$ -Pinene,  $\beta$ -Pinene, 4,8-dimethyl-1,3,7-nonatriene,  $\beta$ -Myrcene, Limonene,  $\alpha$ -Copaene) and two common to *T. macropetala* ( $\beta$ -Pinene e Limonene) a species pollinated by bats (Aguilar-Rodríguez et al., 2014).

Finally, it is important to highlight that the aroma composition varies throughout the day and this issue is important in attracting pollinators (Balao, Herrera, Talavera, & Dötterl, 2011; Aguilar-Rodríguez et al., 2014). Dötterl, Jahreb, Jhumur, and Jürgens (2012) evaluated

the volatile compound dynamics throughout the 24 h in which the flowers were open, observing a variation of these compounds, thus enabling a greater diversity of pollinators, and thus ensuring the reproductive success of the species. In our study, the flowers were collected at anthesis, which occurred between 6:30 and 8 a.m. for all species. Further experiments on the volatile compounds dynamics throughout the day may be interesting for pollination attraction studies in these species.

We identified 71 different volatile compounds, some of them having significant importance in the food, cosmetic, perfume, chemical and pharmaceutical industries. The variation in the odor profile observed of Bromeliaceae in this study shows complex variability. Current taxonomy and pollination syndrome studies can adequately explain the variation in volatile compounds among species. Characterization of these compounds in Bromeliaceae may clarify some problems in taxonomy. Further studies using more species from different genera and detailed morphological information and volatile composition associated with their pollinators can clarify the attraction of pollinators by specific odor compounds.

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## RESUMEN

**Perfil de compuestos volátiles de las flores en las Bromeliaceae.** Los compuestos volátiles tienen un papel vital en el ciclo de vida de las plantas. Poseen actividad antimicrobiana y anti-herbivoría biológica y una gran importancia en la industria de alimentos, cosméticos, perfumes, productos químicos y farmacéuticos. Este estudio tuvo como objetivo identificar los compuestos volátiles de trece flores de especies, pertenecientes a cuatro géneros

de Bromeliaceae utilizando microextracción en fase sólida mediante cromatografía de gases hifenada con espacio de cabeza acoplada a espectrometría de masas. Se han identificado setenta y un diferentes compuestos volátiles pertenecientes a nueve grupos. Los compuestos identificados representaron más del 97 % de los componentes principales en *Aechmea bicolor*, *Ae. bromeliifolia*, *Ae. distichantha*, *Ae. fasciata*, *Vriesea friburgensis*, 99 % en las variedades de *Ananas* y 90 % en *V. Simplex*. *V. friburgensis* mostró la mayor diversidad de compuestos volátiles con 31, mientras que en *Al. nahoumii* se han encontrado sólo 14 compuestos. Las tres variedades de *Ananas* presentan los mismos 28 compuestos en cantidades relativamente similares, lo que se confirmó por el análisis de componentes principales. Estudios taxonómicos y síndromes de polinización disponibles podrían explicar la variación de los compuestos volátiles entre especies.

**Palabras clave:** bromelia, cromatografía de gases, espacio de cabeza, espectrometría de masas, análisis de componentes principales, terpenoides.

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