Applications in Plant Sciences

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PROTOCOL NOTE

INVITED SPECIAL ARTICLE
For the Special Issue: Conducting Botanical Research with Limited Resources: Low-Cost Methods in the Plant Sciences

Low-cost FloPump for regulated air sampling of volatile organic compounds

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PREMISE: We present a low-cost, battery-operated, portable pump, "FloPump," which allows regulated air sampling for the study of volatile organic compounds (VOCs). VOCs are routinely investigated in applications such as atmospheric chemistry, agriculture, and fragrance biology.

METHODS AND RESULTS: We compared the performance of FloPump with the Supelco pump in collecting VOCs using two test samples: guava fruit (*Psidium guajava*) and a perfume. The sampling and identification of volatiles was carried out using a dynamic headspace sampling method followed by gas chromatography–mass spectrometry. We show that the sampling efficiency of FloPump is comparable to the commercial pump, and at an affordable cost of ~US$115 (~86% cheaper), it provides a viable option for researchers interested in sampling volatiles on a constrained budget.

CONCLUSIONS: Accurate air sampling is critical for the study of VOCs. We propose that FloPump will make air sampling more affordable, thus encouraging studies of VOCs.

KEY WORDS dynamic headspace sampling; fragrance analysis; micro air sampler; plant volatiles; regulated air flow.

Volatile organic compounds (VOCs) are a group of organic compounds released by living organisms or human-driven processes. Plants and animals use VOCs for chemical communication, and plants also use them for defense against herbivory and in the production of fragrances for pollinator attraction (Raguso and Pichersky, 1995; Raguso, 2008, 2009; Arimura et al., 2009; Lucas-Barbosa et al., 2011; Schiestl and Johnson, 2013; Duffy et al., 2018). The presence of VOCs has also been proposed as an important indicator of phytochemicals that may be critical in drug discovery (Guo et al., 2006; Togar et al., 2015). In the past two decades, human-made VOCs such as benzene, carbon monoxide, and formaldehyde have been in the news due to their toxic and hazardous effects on human health, especially when they are found in everyday materials such as paint, adhesive removers, tobacco smoke, and stored fuels (Mølhave, 1991; World Health Organization, 2010; Fang et al., 2019). These chemicals are often investigated in assessments of air quality as they are considered diagnostically important in determining air toxicity.

Over the past two decades, the biological community has shown interest in flavor- and fragrance-related VOCs (Raguso and Pichersky, 1995; Raguso, 2008, 2009; Schiestl and Johnson, 2013). These molecules are composed of a wide range of VOCs, including those from linear and cyclic terpenes (e.g., linalool, myrcene, and menthol), esters (e.g., geranyl acetate and methyl butyrate), and aromatic compounds (e.g., benzaldehydes and vanillin) (Schiestl and Johnson, 2013; Byers et al., 2014). The first step of studies focused on the detection of natural VOCs and the regulation of human-made VOCs is to collect volatile compounds from their source. This often involves the collection of air at a regulated, predetermined rate onto an adsorption unit. The collected volatiles are then subjected to extended lab-based analytical methods (such as gas chromatography–mass spectrometry [GCMS]), which aim to identify individual chemical compounds in the sample.

Air-sampling tools often consist of bulky and expensive pumps that are not practical for collecting air samples from remote locations where conveyance is difficult. Small, portable pumps are used in these circumstances, which tend to be expensive (~US$800) and not locally available in developing or underdeveloped countries. In addition to this, each experimental setup may require multiple air-sampling units, which is challenging in field studies in which access to locations and the flowering times of plants may be limited. These combined constraints make VOC studies very difficult in economically disadvantaged countries and limit such studies to researchers who have substantial funds. To enable the collection of VOCs at an affordable cost, without compromising the quality of the samples, we present details of a push-and-pull pump that we have designed and manufactured, which is cost effective, affordable, and portable. The pump is also available for purchase (at the cost mentioned here) for use by interested researchers.

METHODS AND RESULTS

Features of the machine

Because any VOC collection requires a push pump (air blowing) and a pull pump (air suction), our pump is designed with both these features incorporated within the same machine. The final design is called the "FloPump," because we originally built it for floral volatile collection, but generically it represents the "flow" of air both into and out of any VOC-collection system. Most of the features in the FloPump match or surpass those available in the commercial brand Supelco (Sigma-Aldrich, Waltham, Massachusetts, USA), against which the FloPump was tested. All improvements in the FloPump are detailed here and in Table 1. The manufacturing of the FloPump was outsourced to a local startup based in India (SoftCircuits Cloud Innovations Pvt. Ltd., New Delhi, India; http://softcircuitsindia.com/index.html; program included in https://github.com/SoftCircuits-Innovation/FloPump-TrEE_lab [accessed 23 March 2020]; Appendices 1, S1), under a contract.

The FloPump has a durable, lightweight plastic body with a connector (silicone tube with a 4-mm inner diameter) to which a volatile collection trap (VCT) can be attached. Airflow is controlled by a microcontroller, which controls the airflow rate and the total run time. At the end of the set time, the machine shuts down automatically, thereby facilitating unmonitored, accurate sampling across different samples. The airflow can be regulated between 47.19 and 707.92 mL/min by long-pressing button a, which takes the user to the configuration mode. In this mode, buttons b and c can be used to increase or decrease the values, respectively (4 in Fig. 1A, B). Once the desired speed or time is selected, the settings can be saved by pressing button a. Once saved, button c can be pressed to start the pump. All selected configurations can be viewed on the digital display on the front, which makes the use of the machine very intuitive. Options for air push or pull can be regulated by choosing the right outlet in the pump (11, 12 in Fig. 1C), and the two outlets can be switched as required. Finally, the FloPump has a convenient USB charging port (17 in Fig. 1C, D) and can also be charged using a power bank or an in-car charger. The charging mode is indicated by a red light, and the battery mode is indicated by a blue light.

TABLE 1. A comparative chart highlighting features available in the FloPump and a commercial pump (Supelco).

Features	FloPump	Commercial pump (Supelco)
Flow range (mL/min)	47.19–707.92	94.39–471.95
Standard deviation in the flow rate (mL/min)	±23.60	±14.16
Battery type	Inbuilt, rechargeable	External 9-V battery
Timer	Yes	No
Digital display	Yes	No
Cost	US$115	~US$800

The FloPump runs for at least 12 h for lower flow rates (e.g., 165.18 mL/min), and it runs for at least 10 h for higher flow rates (e.g., 500 mL/min). To record variation in the pump, we calculated the standard deviation (SD) in flow rate for the two pumps; the SD value for the FloPump is ±23.60 mL/min (Table 1), which is higher than the flow rate recorded for the Supelco pump (±14.16 mL/min). The two pumps differ significantly in the ranges of their flow rates; FloPump provides a wide flow rate range (47.19–707.92 mL/min), which is superior to the Supelco pump (94.39–471.95 mL/min). Due to the difference in these flow rate ranges between the two pumps, we propose that the SD values be used as a feature of the pump rather than a measure of efficiency of the two pumps. To test the operative efficiency of the FloPump in collecting volatile compounds, we tested it against the Supelco pump; the experimental design and outcome is described in the next section.

A comprehensive list of all the components required for manufacturing the FloPump are listed in Table 2. We also provide a block diagram with the complete circuits for each block in Appendix S1, which provides detailed information on the flow of instructions/functions in the machine by breaking the different components of the machine into smaller sections. The protocol to build the FloPump is also given in Appendix 1. The sale price of the FloPump in India is ~US$115, in contrast to the estimated market price for similar pumps with fewer features of ~US$800. Features such as digital displays, automatic shutdown at predetermined settings, and USB charging are not found in the Supelco pump and are unique to FloPump.

VOC collection and operative performance of FloPump, with GCMS analysis

To test the performance of the FloPump, we compared its collection efficiency with that of the Supelco pump using a dynamic headspace sampling method (Raguso and Pellmyr, 1998; Tholl et al., 2006) (Table 3). VOCs were collected from two sources, a ripe guava fruit (*Psidium guajava* L.) and a commercially available perfume (Maestro; Park Avenue, Thane, Maharashtra, India), both of which are fragrant. The guava fruit was cut into two equal halves (65 g each) and placed into a polyacetate bag (Oven Bags; Reynolds Kitchens, Lake Forest, Illinois, USA) to create a headspace. Similarly, 1 mL of the liquid perfume was spotted onto a filter paper (8 cm^2) and then enclosed in a polyacetate bag.

Using two pumps for each experimental setup, we created a push-and-pull pumping system, where clean air was pumped into the headspace at a constant rate of 350 mL/min, and the headspace air was pulled out of the polyacetate bag at a constant rate of 250 mL/min. We used a VFA series flowmeter (Visi-Float; Dwyer Instruments Inc., Michigan City, Indiana, USA) to calibrate and monitor the flow rate of the push-and-pull pumps. Volatiles from the headspace were collected by connecting one end of the pull pump to a VCT (Volatile Collection Trap LLC, Gainesville, Florida,

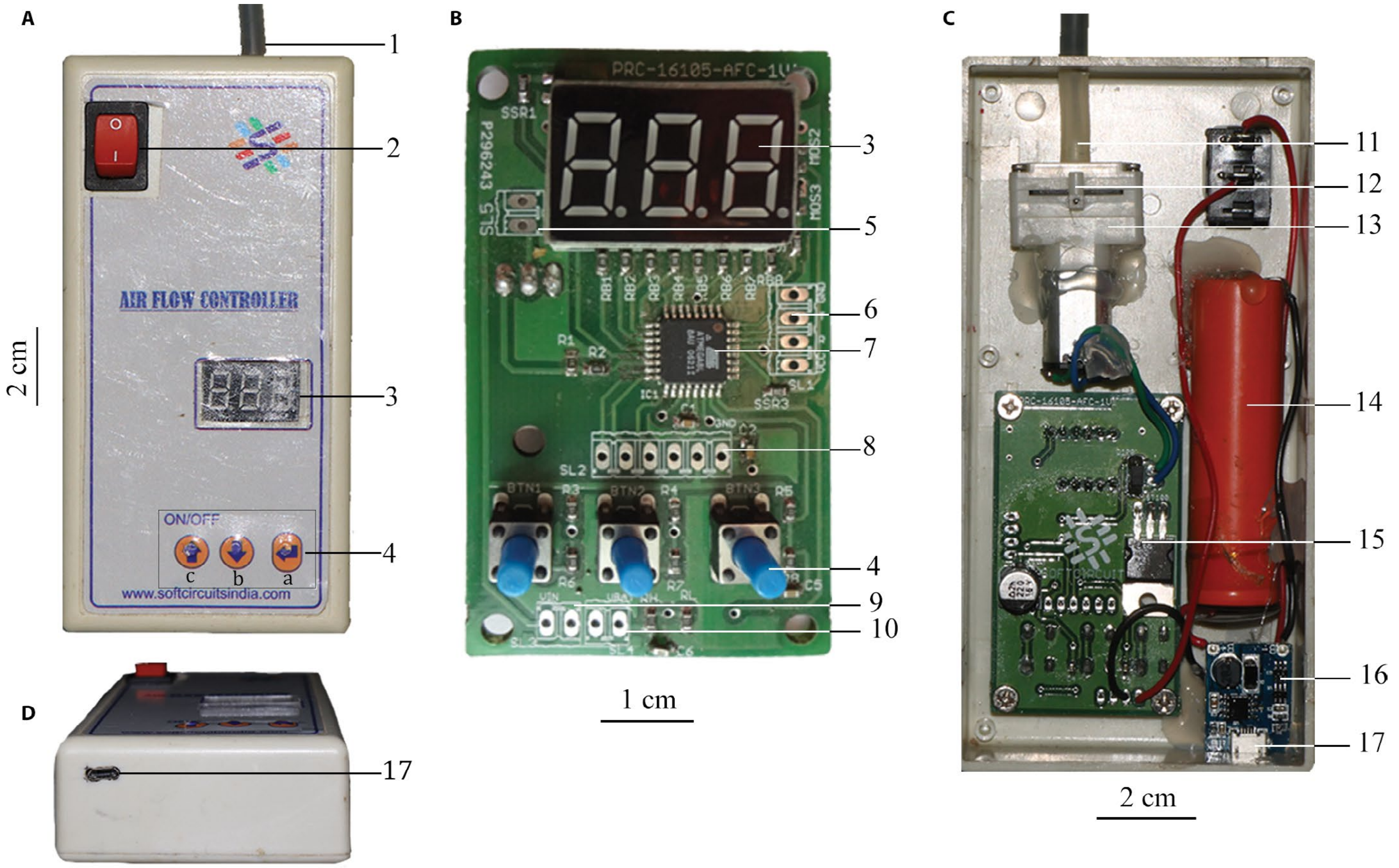

FIGURE 1. The FloPump (A–D) is shown here in front view (A), a close-up of the circuit board (B), with the front cover removed (C), and view of the base (D). The FloPump consists of an air outlet (1) that can be modified for blowing or sucking air (11 and 12), an ON/OFF switch (2), a seven-segment digital display (3), a configuration panel (4), and a USB charging port (17). The configuration panel has three input buttons (corresponding to the tactile switch) for various functions such as setting the time and flow speed (buttons a, b, c). Inside, it has a printed circuit board (PCB, as shown in B and 15 in C), where all the electronic components are connected, including the digital display (3), microcontroller (7), tactile switch (4), motor (13), and a battery (14). The battery has a separate charging circuit (16) and a USB connector (17), which is connected to the PCB via connectors SL_3 (9) and SL_4 (10). On the PCB, SL (5, 6, 8, 9, and 10) represents the connections shown in the schematic diagram in Appendix S1. [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 2. List of the components required to build the FloPump.

Item	Make/Model no.	Quantity
Battery from 2200 mAH[a] power bank with inbuilt charging circuit	Generic[b]	One
DC 5–6-V diaphragm air pump with motor providing the desired rounds per minute	Generic	One
Microcontroller	ATMEGA8L-8AU	One
Seven-segment display	Generic	One
Resistors	Generic	10K[c] (6), 1K (8), 100E[d] (4), 51E (3)
Capacitors	Generic	0.1 μF[e] (5), 1 μF (1), 100 μF (1)
Metal-oxide-semiconductor field-effect transistor (MOSFET)	BSS123	Three
NPN transistor	TIP122	One
Diode	D100	One
Main switch for power	Generic	One
Tactile switch	Generic	Three
Wires	Generic	As per requirement
Universal serial bus (USB) programmer	Atmel corporation	One
Printed circuit board (PCB)[f]	Generic	One

[a] mAH is 10^{-3} Ampere-hour.
[b] The term "generic" refers to components that can be bought from any electronics supplier and is not model or brand specific.
[c] K: $\times 10^3$ and units are in Ohm.
[d] E: $\times 10^0$ and units are in Ohm.
[e] μF: 10^{-6} Farad.
[f] See Appendix S1, and step 1 in Appendix 1 for PCB configuration.

TABLE 3. List of components required for the volatile collection experiment using dynamic headspace sampling.

Item	Quantity
FloPumps with suction (pull) and blowing (push) outlet	One each
Flowmeter (example select range: 0–800 mL/min)	One
Polytetrafluoroethylene (PTFE) tape	One
Packed adsorbent	One per sample
Polyethylene terephthalate (PET) clear bags (oven bags)	One per sample
PET pipes	Two per setup
Twist ties	As per requirement
Charcoal filter	Two per setup
MS-grade solvents (acetone and *n*-hexane)	As per requirement

USA), which contained 20 mg of Porapak Q (Volatile Collection Trap LLC) as an adsorbent. To avoid contamination, the VOC collection was performed under a fume hood at the Indian Institute of Science Education and Research Bhopal (IISER Bhopal), India. Volatiles from the two test sources (guava and perfume) were collected in four replicates for both pumps (FloPump and Supelco), along with a blank control, and the pumps were run for 60 min in each setup. Finally, the volatile compounds were eluted from the VCT using a 300-μL mixture of MS-grade *n*-hexane and acetone (9 : 1), and the eluent was stored in 1.5-mL glass vials at −20°C until its analysis on a Clarus 690 gas chromatograph (PerkinElmer Inc., Waltham, Massachusetts, USA) with a Clarus SQ8T mass spectrometry detector attached.

To compare the air sampling efficiency of the FloPump with the Supelco pump, we used the VOCs in the headspace samples collected from the ripe guava fruit and the commercial perfume brand. We prepared the samples for GCMS analysis by adding 5 μL of 0.05% toluene (in *n*-hexane) as an internal standard to 75 μL of the sample solution (Raguso and Pellmyr, 1998). A 1-μL aliquot of this mixture was injected in the splitless mode on the Elite-5MS GC column (diameter 0.25 mm, length 30 m, film thickness 0.25 mm; PerkinElmer Inc.). Helium was used as the carrier gas at a flow rate of 1 mL/min, and the injector temperature was set to 230°C. The oven program was set to an initial constant injection temperature of 50°C for 2 min, followed by a 10°C per min increase in temperature to a final temperature of 230°C, where it was held for 4 min. We identified individual chromatogram peaks in the total ion chromatogram for each sample by comparing the mass spectrum of each compound with the National Institute of Standards and Technology (NIST) mass spectral library (Fig. 2A, D for the FloPump and Fig. 2B, E for the Supelco pump) (Table 4). A VOC was considered present in a sample if it was absent in the blank control but present in more than one test sample. To quantify the relative abundance of all volatiles in the samples, we calculated the peak area for each volatile using TurboMass software (PerkinElmer Inc.) as follows: (1) area % = 100 × (peak area of a compound/summation of all peak areas), and (2) normalized area % = 100 × (peak area of a compound/largest integrated peak area). The difference in the relative abundance of each compound sampled through the FloPump and Supelco pumps was statistically tested by performing a Wilcoxon rank-sum test (R Core Team, 2018).

For the volatiles collected from guava fruit, both pumps detected two compounds, with no significant difference in the VOC collections of the two pumps (Table 4, Fig. 2A–C). Similarly, in the perfume sample, both the FloPump and Supelco pumps successfully extracted the same eight VOCs; however, the eight compounds were never detected in a single sample run from either of the pumps. A total of seven compounds were present in all samples irrespective of the pump type, although one compound (myrcene) was absent from at least one of the four replicates for both the FloPump and the Supelco pump (Table 4). The Wilcoxon rank-sum test showed no significant difference between the two pumps when the relative volatile abundance was compared using the percent peak area method as well as the normalized peak area method for all eight compounds in the volatile samples (results for percent peak area are presented in Table 4, Fig. 2F), suggesting a similar VOC collection efficiency and operative performance for the FloPump and the Supelco pump.

CONCLUSIONS

The FloPump described here comprises a pump and a microcontroller for controlling the digital display and air flow rates. The FloPump is highly efficient, portable, easy to use, and approximately ~86% cheaper than commercially available pumps. The improved design of the FloPump, particularly the visible display of the flow rate, makes it easy to use without compromising its portability or performance. Furthermore, the addition of a USB charging port makes it easier to charge the FloPump in remote areas using a power bank or in-car charger, which also eliminates pollution from spent batteries. The FloPump can be used in any experimental setup that requires a regulated air flow, such as room air sampling and environmental air sampling. The availability of low-cost machines such as the FloPump can help accelerate research in the field of chemical biology, especially in developing countries where researchers may be financially limited. Our current understanding of VOCs from tropical flora and fauna is very limited and strictly restricted to plants grown in gardens or animals reared in captivity. Reducing the costs of research apparatus, especially for sample collection, can significantly improve the global sampling of volatiles from plants and thus enhance our understanding of plant behavior and communication. We propose that the FloPump can be used to facilitate the establishment of a "VOC bank" in research labs where GCMS facilities might not be immediately available by allowing the freeze-storage of samples collected in the field. These samples can then be analyzed at a later stage when GCMS facilities are made available. We strongly believe that reducing the costs of basic equipment alleviates a major bottleneck in many research fields, making science accessible and affordable to all.

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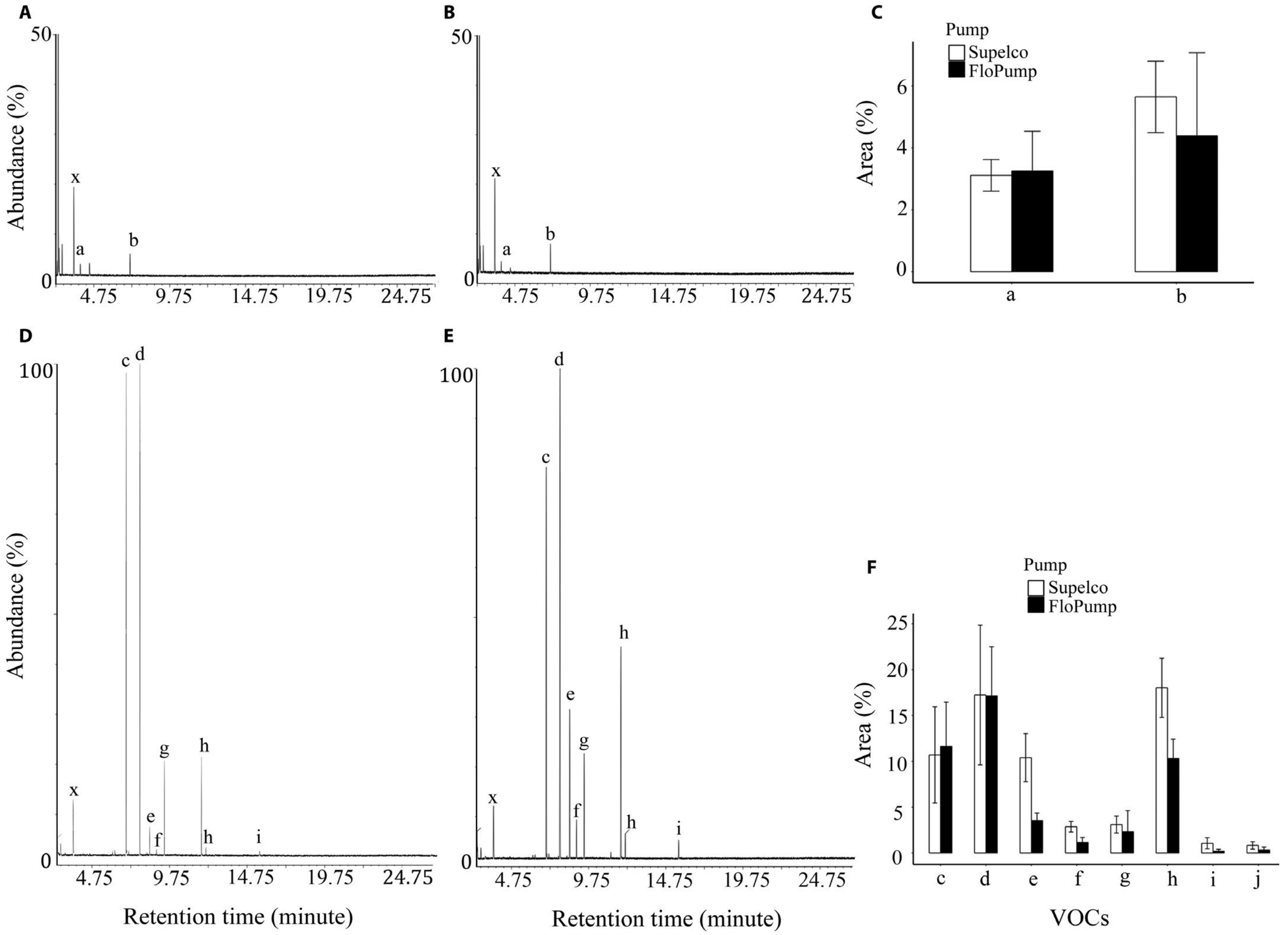

FIGURE 2. Total ion chromatograms used to visualize the volatile organic compound peaks when using a FloPump (A, D) and a commercially available Supelco pump (B, E). Both machines identified two compounds (a, b; see Table 4) in the guava fruit samples (A, B), whereas a total of eight compounds (c–i) were identified in the commercial perfume samples (D, E). The peak corresponding to the internal standard (toluene) is shown as x. The peak areas for all the compounds were measured, and we found no statistical difference between the sampling efficiency of the two pumps (C, F). The compound names (a–i) and the statistical test results are provided in Table 4.

TABLE 4. List of all volatile organic compounds identified from a ripened guava fruit and commercial perfume sample using two air sampling pumps, the FloPump and the Supelco pump, and statistical comparison of their sampling efficiency.

			Wilcoxon test result[b]	
Sample type	**Peak ID[a]**	**Compounds**	***P* value**	**Statistic (W)**
Guava fruit	a	Butanoate	1.000	3
	b	Hexanoate	0.800	4
Commercial perfume	c	3,4-Dihydroisoquinolin-7-ol, 1-[4-hydroxybenzyl]-6-methoxy-	0.850	5
	d	Limonene	1.000	6
	e	Dihydromyrcenol	0.057	12
	f	Linalyl formate	0.228	10
	g	1,3-Dioxolane, 2-hexyl-	0.800	5
	h	Verdox	0.333	4
	i	Phthalic acid, 2-chloropropyl ethyl ester	0.857	6
	j	Myrcene	0.190	16

[a] Chemical peaks detected in the total ion chromatogram shown in Fig. 2. Each peak was identified by comparing the mass spectrum of the molecule with available mass spectra in the NIST library.

[b] The Wilcoxon rank-sum test summarizes the absence of a statistical difference in the relative abundance of each chemical compound detected after being collected using the two air sampling pumps.

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AUTHOR CONTRIBUTIONS

P.S. monitored the machine manufacturing, collected the VOC samples, and performed the GCMS analysis. V.G. conceived the idea and supervised the analysis. P.S. and V.G. wrote the manuscript. Both authors have read and approved the manuscript.

DATA AVAILABILITY

The PCB design for the FloPump is available at Github (https://github.com/SoftCircuits-Innovation/FloPump-TrEE_lab).

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

APPENDIX S1. Block diagram showing detailed circuit connectivity between different parts of the FloPump.

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APPENDIX 1. Detailed stepwise description of how to build the FloPump, followed by a user guide.

Stepwise protocol for building the FloPump

1. The first step is to design and manufacture a printed circuit board (PCB). The PCB design for the FloPump is available from the Github repository (https://github.com/SoftCircuits-Innovation/FloPump-TrEE_lab).
2. Buy the components listed in Table 2.
3. Install Atmel Studio to edit and transfer the code to the microcontroller using the USB programmer.
4. The connection denoted as SL_2 (i.e., “uC_Prog_conn”) on the schematic provided in Appendix S1 can be used to connect the PC and programmer connector on the PCB. The code (provided on the Github repository, mentioned above) can be modified based on the user’s requirements. The current code allows users to set both the airflow speed and the run time of the machine.
5. Assemble the components on the PCB as per the schematic, and connect the battery and pump to the PCB.

Stepwise FloPump user guide

1. Select the appropriate outlet at the top of the machine for air suction or air blowing and connect the flowmeter (see 11 and 12 in Fig. 1C). This step involves the calibration of the FloPump for the desired air flow rate by connecting it to a flowmeter.
2. Press the main ON/OFF switch (see 2 in Fig. 1A) to turn the FloPump ON. Press button c to start the pump.
3. Long-press button a to set the flow rate. Press buttons b and c to decrease or increase the air flow rates, respectively (see 4 in Fig. 1A, B). Press button a to set when the desired flow rate is reached.
4. Repeat step 3 to set the air-sampling duration using the same procedure as was used to set the air flow rates. This completes the calibration for air flow rate and the configuration of the runtime for the FloPump. Remove the flowmeter.
5. Replace the flowmeter with the connection tubing linked to the head-space sampling unit designed for the collection of VOCs. Press button c to start VOC collection using the FloPump.
6. The stepwise VOC collection setup from pump to source may be designed as: FloPump → charcoal filter → VOC collection tubes → headspace. Each connection here is represented by an arrow, which in reality should be an inert silicone tube.