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Sniffer bees as a good alternative for the current sniffing technology

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ABSTRACT
Various kinds of sensors or biosensors have critically been reviewed, especially for their advantages and limitations. Electronic nose and canines are the most commonly used sniffing devices in the market. However, these odor sensors do not always provide reliable results. Electronic nose has limitations in terms of its selectivity and sensitivity, whereas canines are easily disturbed by distractions, thus giving unreliable results. Ambient mass spectrometry is a favorable technique in odor detection, but it is still limited in usage due to its high cost and universality. On the other hand, insect-based sensors, especially sniffer bees are rising up to be a new sniffer technology nowadays. The performance of sniffer bees was found to be better or at least comparable with sniffer dogs in terms of number of targeted odorant and sensitivity limit. Sniffer bees are highly sensitive, cost-effective and portable for field operation. They are easy to be conditioned to detect target odorants using sugar solution as a food reward during classical Pavlovian conditioning training. The natural food forging behavior of honey bees through the proboscis extension reflex is captured and further processed to be informatic signal during data interpretation. Nevertheless, the application of sniffer bees are still not popular and acceptable as compared to other sensors. There are few research groups actively involved in this study. Further studies are on-going to improve this kind of biosensor, so that it can be applied to wider applications including pharmaceuticals, food safety, forensic and country security.
Keywords: *sniffer bee,biosensor, Pavlovian conditioning, proboscis extension reflex, olfactory system*

1. ODOR DETECTION AND MEASUREMENT

Odorants are small volatile molecules that have high vapor pressure at room temperature, and they usually present in very low concentration down to parts of billion or trillion level [1-3]. They are considered as chemical stimuli which can be perceived by human and animals with the sense of olfaction. The stimuli are rich in information, and interacting with olfactory receptor neurons in the olfactory epithelium lined the interior of nose. The olfactory system processes the information about the object identity, concentration, and quality of the chemical stimuli. The olfaction of human is often considered the least acute of the senses, and many animals are apparently superior in their olfactory abilities. This difference is probably due to the larger number of olfactory receptor neurons in the olfactory epithelium of animals, and therefore, providing larger area of cortex devoted to olfaction [4].

Indeed, human olfactory system has advantages over chemical and instrumental methods in terms of sensitivity and flexibility. The human olfactory system is more sensitive than the current chemical and instrumental methods such as gas chromatography integrated with mass spectrometer and electronic nose. The human olfactory system is able to detect and identify a wide variety of chemicals and giving different responses. In contrast, chemical and instrumental methods are usually restricted to limited number of chemicals and giving a similar response to all compounds.

Although the human olfactory system is likely to be better "sensor" than chemical and instrumental methods, this biosensor has also some shortcomings. The use of human olfactory in odor detection and measurement usually produces variability. The variability is mostly due to human moods, biases and other vagaries. This is because each individual is different in intelligence, sensitivity, experience, persistence and interests. These differences make the response of individuals to be inconsistency [5]. Several complementary methods are being explored to further investigate for alternatives to assist human beings to obtain reliable data in odor detection and measurement since the last century.

2. RATIONALE OF HONEY BEES AS SNIFFERS

Honey bee possesses several key criteria to be an excellent sniffer. In particular, the bee species of *Apismellifera* which is the subset of bees in the genus Apis has been investigated for its capacity in odor detection and recognition. Honey bees have a pair of mushroom bodies and higher number of odorant receptors (170) in the antennae which have equipped themselves to be excelled in olfactory learning and memorizing. Their response for food uptake by proboscis extension could be used as a signal to be captured during sniffing training. This behavior of extending proboscis to take food when they are exposed to target scent will make them to be trainable as sniffer bees. They have outstanding ability to distinguish scent with the sensitivity down to parts per trillion level [6].

The Defense Advanced Research Projects Agency (DARPA) under the United States Department of Defense funded a project in 2004 to utilize honey bees for explosives detection. It was found that they could detect trinitrotoluene (TNT) at the level of parts per trillion [7] and 2,4-dinitrotoluene (DNT) down to at least 78 ppt[8]. Under the project, scientists from a biotechnology based company, Inscentinel Ltd., United Kingdom worked together with researchers from the University of Montana and the US Department of Energy's Los Alamos National Laboratory [9]. Inscentinel Ltd specializes in the development of insect olfaction technologies for the detection of trace chemicals. The company also collaborated with another company, Panchromos Ltd. to invent a hand-held, battery-powered, bee-holder and detector for field testing named as Vasor136 (Volatile Analysis by Specific Odour Recognition) which can house 36 bees to detect 6 different chemicals simultaneously. Based on their studies, 6 to 18 sniffer bees are adequate to generate reliable data in a biosensor. The prototype appears to be suitable for a limited number of target odorants from drugs and explosives only. The reliability, accuracy, stability and sensitivity of the prototype are still under investigation. They highly believe that all these parameters can be affected by the factors of climate, bee species and target odor.

The sniffing capacity of honey bees has also expanded to medical and agricultural industries recently. According to Suckling and Sagar [10], honey bees can be trained to detect the scent of Mycobacterium tuberculosis from human breath. A person who is suffering from Tuberculosis can be diagnosed in a faster and more sensitive manner using sniffer bees. In agricultural industry, honey bees were successfully used to detect the presence of Mediterranean fruit fly (*Ceratitiscapitata*Wiedemann) larvae in Valencia oranges at the early stage of maturity [11]. Therefore, honey bee could be the best sniffing device or sniffer model since the cost of training and maintenance for honey bees are lower than sniffer dogs, without compromising the sniffing performance in terms of accuracy and sensitivity.

2.1. Mushroom bodies for olfactory learning and memorizing.

Apismellifera is physiologically suitable to be sniffer bee. Each bee has a pair of structures called mushroom bodies in the head. The mushroom bodies are the center of insect brain for learning and memory [12-14]. This structure has two distinct compartments, namely a set of intrinsic neurons as Kenyon cells, and associated neuropils formed by the processes of the Kenyon cells [12, 14, 15-18].They integrate different inputs and create novel association which help them in learning and memorizing [19].

Erber et al. [20]reported the importance of mushroom bodies for bees in olfactory learning by local cooling experiment. They used small metal probe to cool the mushroom body calyces (or antennal lobes), and it caused impairment to the formation of olfactory memory in honey bees. Another experimental evidence was provided by Voskresenskaya [21] who demonstrated the importance of mushroom bodies in olfactory memory. He performed the experiment by removing the mushroom bodies surgically from the trained honey bees. As a result, a complete loss of conditioned response was observed in the honey bees. The memory of sniffer bees will last for days if they are exposed to a single odor associated with sugar reward. Interestingly, lifelong memory can be promoted if they are exposed to an odor associated with sugar reward for 3 times [22]. The observation supports the finding of Menzel and Erber [23] who reported high probability of bees to correctly identify the odor associated with sugar reward.

2.2. Odorant receptors in antennae.

Honey bees sense and locate hive intruders by their antennae. The antennae are a pair of sensitive receptors. Its base is located in the small socket-liked membranous areas of the head wall. The antennae can move freely in every direction and have thousands of sensory organs. Their functions are to feel or touch (mechanoreceptors), to taste (gustatory receptors) and to smell (odor receptors), and therefore to guide the honey bees to distinguish the floral and pheromone odors. They are able to locate flowers (food source) and communicate with other bees by using antennae. In particular, the foraging worker bees manage to discriminate and choose flowers among the flowers they visited by using subtle olfactory cues [24]. Odor molecules are collected by the olfactory sensillae of bees which can be found in their antennae. The odor molecules bind with specific proteins in antennae and being collected to a membrane-bound receptor on a nerve cell.

The antennae of honey bees are the olfactory receptors. Karl von Frisch (1886–1982), an Australian zoologist who devoted his life in the study of bees, discovered the role of antennae [25]. He found that worker bees could be trained to visit dishes containing odor of flowers. When the antennae were surgically removed, this discrimination ability was totally disappeared. There are 170 odorant receptors in the antennae of bee [24], but only 62 odorant receptors in fruit fly [26-27] and 79 odorant receptors in mosquito [28]. The antennae of bees can also recognize and detect the location of odorant, simply based on the intensity of odorant molecules[29]. This was demonstrated in an experiment conducted for the trained worker bees in a Y-shaped maze. The worker bees were able to choose the correct path toward the location of the odorant. However, the worker bees chose the wrong path when their antennae were glued in crossed direction. Therefore, bees could be an excellent sniffer compared to other insects. Honey bees are superior to be trained as a sniffing device since they have more odorant receptors than other insects.

2.3. Proboscis Extension Reflex in classical Pavlovian conditioning.

Proboscis is the tongue of a bee. It is long, slender and hairy. It acts as a straw to bring liquid food (nectar, honey and water) to mouth. Bee will unfold its proboscis to form a long tube to suck liquid food when taking food. It is folded into mouth cavity in a Z-shaped pattern when unused [29]. The proboscis of honey bee is not a permanent functional organ, it is improvised temporarily by assembling parts of the maxillae and the labium.

Classical conditioning is a form of learning in which the conditioned stimulus (CS) serves as a signal to the occurrence of an unconditioned stimulus (US). A stimulus is a factor that will cause a response from an organism [30]. The US is normally a stimulus such as food or pain that will trigger response, which is known as an unconditioned response (UR). CS usually does not trigger any response in the organism when it is presented alone at the first time. After conditioning, CS will trigger response and it is called as a conditioned response (CR).

This kind of learning technique was reported by Pavlov et al. [31]. In his experiment, he presented the dog with a ringing bell followed by food feeding. The food elicited the salivation of the dog. After several trials, the dog salivated, even though only the ringing bell was presented alone. In this case, the ringing bell is CS, while the food is US. Food triggers salivation, and this response is considered as UR. The dog produced saliva after exposed to CS alone and this response is regarded as CR.

The similar paradigm can also be applied to honey bees. This classical conditioning has been proven to be applicable for honey bees as reported by Bitterman et al. [32].The flow diagram of this approach is presented in Figure. 1. A honey bee is put comfortably at the rest condition. The bee is then exposed to an odor (CS) for 2 seconds and subsequently followed by touching its antennae with a stick dipped with sucrose solution (US). The bee extends its proboscis for the feed after the antennal stimulation. The bee is immediately fed with sugar solution to complete the first cycle of bee training. After several cycles, the bee has associated the odor with sugar reward and it will show proboscis extension reflex (PER) even in the absence of sugar reward. The bee is successfully trained to be sniffer bees by associating US with CS to elicit PER. The whole process is known as the method of classical Pavlovian conditioning. The response of proboscis extension will be captured as a signal for the detection of target odorant. Therefore, the proboscis extension of sniffer bee will be used to detect the presence of target odorant. The probability of honey bees to evoke their PER responding to the CS could achieve up to 90% [32].

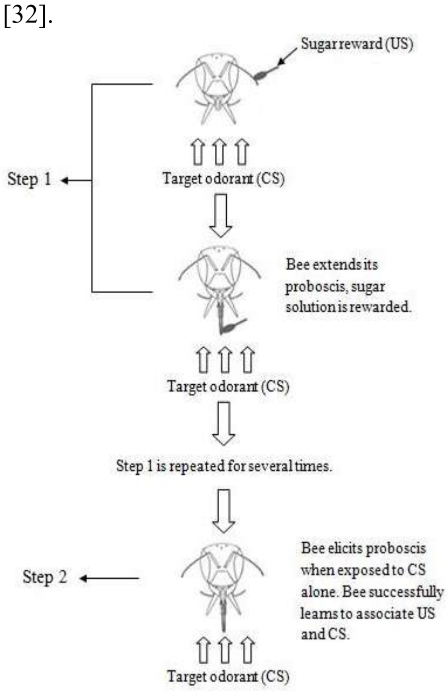

Figure 1. Classical Pavlovian conditioning of honey bee

PER is the nature of bee's feeding behavior. In other words, PER is a response of bees to stick out their tongues when their antennae receive stimulation [33]. PER is evoked when sugar solution is touched to the bee antenna. This reflex response can be used as a signal to study the presence or absence of target odorant. In this case, honey bees must be restrained in a comfortable zone, so that they can perform well without panic, in order to obtain high accuracy of data. The honey bees are positioned in such a way that their heads can easily protrude out of the bee holders. Their heads are allowed to move freely and their responses to the target odor can be observed clearly.

Surprisingly, Letzkus et al. [34] reported that there are more olfactory sensilla in right antenna than left antenna of honey bees. The right antenna could respond better in the associative learning than the other antenna. When one antenna was exposed to the odorant and left the other antennae covered with silicon sleeve, it was found that bees with their right antennae exposed to the odorant could perform better in associating the target odor with food reward.

2.4. Food foraging behavior of honey bees.

Honey bees are fed by pollen and nectar collected from blooming flowers and secretion from other members in the colony. The nectar is stored and concentrated by bees through the processes of evaporation (by wings) and regurgitation to be honey as food for the whole colony. The foraging worker bees are responsible to collect food for the whole colony. They forage to the blooming flowers, sucking up nectar, and then storing in the anterior section of their digestive tracts. They collect pollen by grooming off from their bodies onto the pollen baskets on their hind legs. They can visit 50-100 flowers in a trip and it is about 10 trips in a day [35]. They forage for food every day from 10 am to 2 pm, except raining day, high wind day or the day with extreme temperature (>46˚C) [36]. They will return to their beehive immediately if the light intensity of the day changes rapidly. Therefore, worker bees are well known as the most hardworking insects.

This food foraging behavior of honey bees has been explored to trace the location of odorant as free-flying bees. The bees which have been conditioned to detect specific chemicals or drugs will move toward the direction of target odor during food foraging. For instance, honey bees were trained to detect landmines by previous researchers [6]. Sniffer bees were trained to execute this task which is a very difficult and dangerous duty to track precisely the location of target odorant. However, the limitation is in videotaping which is not practical because of the light body weight of bee (100-120 mg), limited resolution and range of camera.

Recently, a method named 'light detection and raging' (LIDAR) measurement has been applied to track the location of bees. LIDAR is a remote sensing technique that uses laser light to measure distance by illuminating a target. This method works by transmitting laser light pulses over the area where bees are trained to fly to a particular target. The laser light that strikes the bees is scattered back to a detector collocated with the laser. The time between the outgoing laser pulse and the return signal is used to measure the distance from the bees to the LIDAR. A map of the location of the bees can be produced by using a narrow laser beam and scanning this beam over time. The LIDAR system can provide both the range and coordinates of the target so that the location of buried landmines can be accurately mapped [37].

3. INSECT BASED SNIFFERS

Insects such as bees, wasps and moths can be trained to be sniffer device due to their high sensitivity of the olfactory senses. The trained hymenopterans were reported to successfully detect explosive materials including trinitrotoluene (TNT), Semtex, and C-4 (plastic explosive), as well as gunpowder and propellants [38]. However, the performance of sniffer wasps is not comparable to sniffer bees in term of sensitivity. Sniffer wasps are only able to detect scent down to parts per million level [39]. According to Olson et al. [40] , the parasitic wasp, namely *Microplitiscroceipes* can be conditioned to associate an odor with its larval host. The wasp was conditioned to deposit an egg in the host when exposed to an odor. The unconditioned stimulus for that case was the host feces. Wasps were conditioned to associate one odor (odor 1) to food and could also be conditioned to associate another odor (odor 2) to its host. As a result, when the wasp was exposed to one of the odors, it would exhibit a resource-dependent behavior indicating which odor was detected. In this manner, the wasps will exhibit food-searching behavior if exposed to odor 1, when exposed to odor 2, they show host-searching behavior [39]. Previously, researchers conditioned wasps to detect odors associated with food toxins [41], explosives [42], and plan odors [43].

The conditioned *M. croceipis* will live for about two to three weeks as adults, and they can remember the odors for at least 48 hours without food [44]. The wasp will associatively learn the current odor which is encountering when provided with food, and its association with previous odor is then diminished. This species is extremely flexible in its ability to sense different compounds, with the sensitivity of 10^{-7} mol/liter for some odors [43]. In a previous study, this species was proven its capability to distinguish different isomers of six-carbon alcohols on the basis of the position of the alcoholic group, and they were also able to differentiate between 1-hexanol and 1-hexanal [45]. Rains et al. [39] developed a portable device and exposed air samples to the conditioned wasps. They observed and interpreted the food-searching behavior of the five conditioned wasps held in a cartridge. The device is named as 'Wasp Hound' and the presence of 3-octanone in some corn samples which is used as feed for livestock can be detected at parts per million levels.

Moth is an insect related to butterfly and it is from the order of Lepidoptera. Although sniffer moths have almost similar sensitivity with sniffer bees, their size are bigger than bees. The device built to accommodate sniffer moths will be larger than bee-based sensor, and thus making it less portable. Moths were also trained to be sniffers for the detection of plastic explosives [46]. In addition, moths were trained to detect cyclohexanone through the classical conditioning, and a voltmeter was used to detect spikes in the signal from the feeding muscles of moths [47]. A device has been developed to hold 10 noctuid hawkmoths, the feeding response of each moth is measured by electromyography [47]. Five moths were conditioned to the target chemical, and another five were used as an unconditioned control to determine whether the responses from the conditioned moths were false positive. This device is reliable for the detection of chemicals associated with explosives, but it is less portable due to its large size (17 kg and $0.25m^3$) [39].

The antennae of honey bees (*Apismellifera*) have finely tuned vapor sensors with the sensitivity threshold down to parts per trillion [48]. Since honey bees have higher number, 170 odorant receptors [24], they have been used to detect explosives in old landmines [6]. They can discriminate more odors than other insects. The adult bees have about one to four months of life and they can retain the learned odors for several days only. With the multiple conditioning trials of the target odors, they might retain the odor memory up to their whole life [23, 49].

The training for sniffer bees is time-saving compared to training for sniffer dogs. It is also easier to condition them to detect target odorants [39]. The process needs just few hours to train hundreds of bees, whereas a period of 3 to 4 months is needed to train a sniffer dog. Moreover, bee training process is cheaper as the food required for the training is just sucrose solution. No experience and skillful handler is needed in the bee training process, but professional handler is highly required to train a sniffer dog. The training for a sniffer dog needs around 73,000 US dollar in the first year and 50,000 US dollar in the subsequent years [50].

Furthermore, sniffer bees will not be easily distracted, while performing their sniffing task because they will be stationed in a dark box. This external distraction should be taken into consideration if higher accuracy of results is required. Meanwhile, sniffer dogs may be distracted while doing their jobs, especially when something is more attractive showing up suddenly. Another important criterion is bees are smaller in size and this makes them more portable than sniffer dogs.

The unique characteristics of honey bees make them to be ideal as sniffers in many applications, but there are still some limitations for bee-based sensor. One of the limitations is the short lifespan of honey bees. Usually, worker bees are chosen to be trained as sniffer bees due to their high sensitivity. However, the lifespan of worker bees is short; they can live up to six to seven weeks only. Therefore, the training procedure must be repeated frequently in order to train new batch of sniffer bees. Another limitation is the fear of human against bees because of their stings. This kind of phobia may limit the application of the bee-based sensor in medical and food industries.

4. ANIMAL BASED SNIFFERS

Dogs have long been exploited as an excellent sniffing device, especially in the airports for security check. Usually, a sniffer dog is trained to work by using its sense of smell to detect substances such as explosives, illegal drugs, or blood in the airports. Sometimes, sniffer dogs are trained to sniff specific substances such as animals in conservation settings, human remains, crime evidence, currency, drugs, explosives, firearms, mobile phone in prison, molds, agricultural items, polycarbonate optical discs and termites. The sniffing capacity of dogs ranges from parts per billion to parts per quadrillium dependent on the kind of subjects. The detection limit for each odor of interest also varies differently. Therefore, validation study is usually carried out prior to site operation. Sniffer dogs are also able to distinguish target scent even though the scent is masked by other odors. Sniffer dogs can detect blood, although the stain has been scrubbed off from the surface.

The performance of sniffer dogs may also reduce if their body temperatures are increased. This is because they do not possess sweat glands and panting is the only way for them to lower down their body temperatures. Since they are not able to sniff or pant simultaneously, a decrease in sniffing rate will occur. This observation had been proven by Gazit and Terkel [51] who compared the ability of sniffer dogs to detect small explosives charges under two situations, namely (1) relaxed and only lightly panting and (2) exercise on a treadmill and therefore heavily panting. It was found that there was a significant decline in the efficiency of sniffer dogs with the increase of panting. It is believed that sniffer bees have the potential to be a complimentary approach to sniffer dogs, particularly in food quality assurance which is less appropriate to be performed by sniffer dogs. Sniffer dogs are likely to be welcomed by Islamic countries for security check at the immigration points such as ports and airports.

Recently, this non-invasive method has been expanded to medical diagnostics. This method is used to detect the volatile compounds of cancer tumors through the breath and urine of patients. Sniffer dogs were trained to detect bladder cancer through the urine odor with a success rate of 41 % [52]. However, the better promising results could be achieved by sniffer dogs to detect lung cancer with the sensitivity and specificity of 99 %, whereas the sensitivity of 88 % and the specificity of 98 % could be obtained for breast cancer detection [53]. Somehow, another study reported that only 71 % of sensitivity and 93 % of specificity for the detection of lung cancer by sniffer dogs [54].

Likewise, rats are trained to sniff smell because of their exceptional sense of smell. A social enterprise (APOPO) studied, developed and implemented the rat detection technology for humanitarian purposes such as landmines and Tuberculosis detection. Poling et al. [55] used the trained rats to detect landmines with the false alarm of 0.33 per 100 m^2. This result had met the requirement of United Nations Mine Action Service [56] for the accreditation mine-detection animals in which animals cannot emit more than two false alarms in a single test search of a 400 m^2 area.

Similarly, rats have also been trained to detect Tuberculosis. A research was conducted in 2012 to investigate the odor volatiles of Mycobacterium Tuberculosis which could be detected by rats. The reference microorganisms such as Mycobacterium Tuberculosis, nontuberculous mycobacteria, *Nocardia sp*., *Streptomyces sp*., *Rhodococcus sp*., and other respiratory tract microorganisms were used for performance comparison. The sensitivity of rats for typical Tuberculosis-positive sputa was 99.15 % with 92.23 % of specificity and 93.14 % of accuracy [57]. The greatest advantage of using rats as a sniffing device is cheaper cost to keep and train rats than dogs.

Indeed, animals and insects are superior to electronic nose in terms of sensitivity and selectivity due to the presence of odorant receptors in their olfactory systems. Moreover, animals and insects sniffers are not easily affected by temperature and humidity changes of surrounding area. Most importantly, they do not response to the “background gases” like water vapor which could make the difficulty in discriminating and interpreting gas samples.

5. APPLICATION OF ELECTRONIC NOSE

The idea of “electronic nose” was introduced by Persaud and Dodd in 1982. They used three different metal oxides as gas sensors and identified several substances by the steady-state signals of the sensors. The development of electronic nose is to mimic the mammalian olfaction to discriminate complex odorant mixture [58].

An electronic nose usually consists of a multisensor array, an information-processing unit, software with digital pattern-recognition algorithms, and reference-library databases. The sensor array is made up of several sensors which are tuned to detect different chemicals. When the sensors are exposed to a complex odor formed of many chemicals, each sensor responses differently to the odor and form a distinct digital response pattern. Therefore, the identification and classification of an odor can be accomplished through the recognition of the unique aroma signature via the collective sensor responses [59].

The application of electronic nose has benefited a wide variety of industries for quality control of raw and manufactured products [60, 61], process design [62, 63], freshness and maturity (ripeness) of fruits [64, 65], shelf-life investigation [66, 67], authenticity assessment of premium products [68, 69], classification of scents and perfumes [70], microbial pathogen detection [71, 72] and environmental assessment studies [73, 74].

Electronic nose can only detect particular or specific gas phase analytes due to its limitation in the selectivity and sensitivity of the sensor arrays [59]. It is not highly efficient to identify any gas sample, but depending upon the required operating conditions of the sensor arrays and the composition of the gases. Most electronic noses are still coupled with other instruments such as gas chromatography to process and analyze data in order to get desirable results. This has limited its application because instrumentation operation and data interpretation are strongly required.

Electronic nose is also known to have lower reproducibility, longer time to recover between samples, and the result is easily affected by humidity and temperature changes [59]. Therefore, electronic nose cannot replace the advanced analytical technology of gas chromatography mass spectrometry (GC-MS) or experienced odor panels in sniffing smell.

5.1 Electronic nose in food quality control.

The recent application of electronic nose is to determine the fruit maturity stage. A study was conducted by Brezmes et al. [65] who investigated the possibility of an artificial olfactory system as a nondestructive method in fruit maturity measurement. The cultivars chosen for the study were peaches, nectarines, apples, and pears. The fruit samples were also characterized using fruit quality techniques in order to make an objective comparison. The techniques tested the fruit quality based on the fruit appearance (size, shape, color and defect), tactile characteristics (firmness), internal characteristics (sugar content and acidity) and vapor production. They reported that principal component analysis (PCA) on the measurement of electronic nose could well predict the optimal harvesting date of peaches and nectarines. The results were in good agreement with the data obtained from the food quality techniques. Similarly, the use of electronic nose could classify pear samples based on their ripeness state after cold storage and shelf-life period. The classification of PCA differentiated the fruit samples that stored for seven days from those samples stored for four and less than four days. The electronic nose also showed a promising result by correlating the electronic nose signals and the firmness, starch index, and acidity of fruits [65].

Few years later, Borah et al. [75] reported the high reliability of neural network based electronic nose system to segregate tea samples according to their aroma quality. In their studies, the electronic nose system which was comprised of an array of four tin-oxides as gas sensors sniffed thirteen randomly selected tea samples from eight categories of tea grades. The result showed high accuracy of classification (> 90%) in term of their aroma profiles. Therefore, this neural network based electronic nose system could be used to monitor the tea quality during tea grading process.

5.2. Electronic nose in disease diagnosis.

Interestingly, electronic nose could be used to solve the problem faced by modern medicine in disease diagnosis, particularly at the early stage of inflammation. This advancement of electronic nose could also reduce the invasiveness in the current diagnostic treatment, thus increasing the probability of recovery. For instance, Bruno et al [76] successfully showed that electronic nose was able to differentiate patients affected by chronic rhinosinusitis from healthy subjects by just examining nasal out breath. They analyzed the intensity and quality of the odorous components in the air exhaled by patients affected by rhinosinusitis using an electronic nose integrated with gas chromatography and surface acoustic wave analysis. There were six peaks, which were not present in the control group, detected in the gas chromatogram of the pathologic subjects.

Recently, electronic nose was applied to detect lung cancer through human breath as reported by Tran et al. [77]. The researchers have proven the measurement of volatile organic compounds from the exhaled breath of lung cancer patients could be used to diagnose early stage of lung cancer. This non-invasive method produced results with the significant difference in the smell-print patterns (gaseous markers) of newly diagnosed lung cancer patients and control subjects in their pilot scale breath analysis.

5.3. Electronic nose in monitoring chemical process.

Electronic nose can be utilized to monitor chemical processes. This can be proven from the study conducted by Rajamaaki et al. [61] who investigated the effectiveness of electronic nose to monitor the composting process. The electronic nose was able to differentiate the composting bins after 13 days of composting supplied with sufficient (optimal aeration) and insufficient aeration.

6. MASS SPECTROMETRY AS ION ANALYZER

Mass spectrometry is an analytical technique in which its basic principle is to generate ions from organic compounds, separate these ions according to their mass-to-charge ratios (m/z) and then detect them quantitatively and qualitatively [78]. Ambient mass spectrometry is a rapid growing analytical technique in recent years. This technique directly analyzes the surfaces without pre-treatment, filtration and extraction, thus speeding up the analytical works [79]. The most popular and reliable techniques in ionization are desorption electrospray ionization (DESI), direct analysis in real-time (DART) and extractive electrospray surface ionization (EESI).

Desorption electrospray ionization (DESI) is an ambient desorption technique developed by Takáts and colleagues [80]. The working principle of this technique is to direct the electrically charged droplets at the ambient object of interest in which ions are released from the surface and then vacuumed through the air into a mass analyzer. It can be used to detect explosives in trace amount on a variety of ambient surfaces within 5 seconds and no sample preparation is required [81]. This technique is also utilized for rapid analysis of pharmaceutical drug formulations [82, 83], biological analysis [84, 85], environmental analysis [86] and forensic analysis [87].

Direct analysis in real-time (DART) was developed by Cody and co-workers [88]. The principle of operation for this technique is based on the atmospheric pressure interactions of long-lived electronic excited-state atoms or vibronic excited-state molecules with the sample and atmospheric gases [88]. DART can be employed in explosives detection [89], food quality and safety analysis [90], forensic analysis [91], analysis of flavors and fragrances [92], environmental analysis [93] and pharmaceutical analysis [94].

Extractive electrospray surface ionization (EESI) is an electrospray-based technique developed by Chen et al., [95]. It has two separate sprayers in which one is to nebulize the sample solution, whereas the other is to produce charged micro-droplets of solvent. Liquid–liquid extraction occurs between the colliding micro-droplets. The compounds of interest are extracted from the analyte solution into the solvent spray in a continuous automatic fashion with no sample preparation steps [95]. It is employed to monitor the maturity and quality of fruits [96], dairy products safety analysis [97] and quality classification of perfumes [98].

Mass spectrometry can be coupled with other instruments for detection and analysis. The most common combination is gas chromatography coupled with mass spectrometry (GC/MS). GC/MS is composed of gas chromatograph and a mass spectrometer. The gas chromatograph consists of a capillary column for compounds separation. The utilization of capillary column in gas chromatograph depends on its dimensions (length, diameter, film thickness) and phase properties of the column. As the sample travels along the column, the difference in the chemical properties of analytes in a mixture will be separated accordingly. The molecules elute from gas chromatograph at different retention times. This allows the mass spectrometer to break the molecules into ionized fragments and detect them individually based on their mass-to-charge ratios [99].

GC/MS can be applied for food flavor studies. Shimoda and Shibamoto [100] isolated and identified the headspace volatiles from brewed coffee with an on-column GC/MS method. Arora et al. [101] studied the aroma characteristics of some volatiles extracted from Cheddar cheese headspace and their impact on the overall Cheddar flavor by GC/MS method. GC/MS is also utilized in environmental assessment, for instance, Davoli et al. [102] characterized the odorants emissions from landfills by solid-phase micro-extraction (SPME) and GC/MS. They evaluated the efficiency of a scrubber plant in the landfill in order to remove unpleasant compounds.

Although ambient mass spectrometry provides benefits for rapid analysis and little sample preparation or pre-treatment is required, there are still limitations found in this technique. The accuracy and precision of this technique are not as satisfying and promising as the hybrid techniques of GC/MS or LC/MS in quantitative analysis. However, sample preparation is required for GC/MS analysis. Ambient mass spectrometry is not a universal tool for detection because of high cost instrumentation. DESI applications are limited by solvents, while DART applications are limited by small molecules [103]. A major disadvantage of EESI is the limitation to analyze a mixture of compounds. The apparatus tends to be over-contaminated with residues from previous experiments.

7. FUTURE PERSPECTIVE OF SNIFFER BEES

Sniffer bees could be the best alternative to perform tasks that are difficult to be carried out by human because of the danger, capability and capacity. Sniffer bees can detect, differentiate and locate the scent at least down to parts per trillion level. Sniffer bee is likely to be the rising star in the sniffing technology, mainly due to the cost effective of training materials and process, as well as its high sensitivity level. Indeed, the sniffing capability of honey bees was started to be inspired by public after a team of researchers from United Kingdom who patented their findings many years ago [9].

Previously, many studies have been carried out to use sniffer bees in the defense system, security, medical and food quality assessment. However, there is no study on the application of sniffer bees to recognize herbs and/or herbal products till to date. To the best of our knowledge, some herbs are precious materials, and thus possibly, people would adulterate a particular herb with other cheaper source of plants. This adulteration is very difficult to trace if the herbal plants have been dried and ground into powder. In this case, sniffer bees might be able to recognize the dried and ground herbal plants effectively compared to human sense and instrumentation. Intensive studies are actively carried out by Malaysian researchers with the aim to prevent local herb smuggling since Malaysia is rich in herbal varieties including herbs with significant pharmacological properties. Sample pre-treatment is always required to turn the solid sample into liquid before testing and analysis using instrumentation. This preparation procedure could be very time consuming and costing, in addition to the effectiveness of extraction method. Therefore, a rapid and accurate method to inspect the quality of raw material before processing is of great importance in herbal related industries. Furthermore, sniffer bees could be the best of choice, especially in food quality assessment. The development of this technology is foreseen to be widely welcomed, particularly by Islamic countries.

8. CONCLUSIONS

Sniffer bees are likely to be a useful sniffing device for odor detection in numerous applications including medical, food safety and even in country security protection at the immigration points. The potential application of sniffer bees could be due to its high sensitivity, efficiency, reliability and cost effective alternative in maintenance and training process. Further scientific and engineering studies should be carried out to ensure high number of sniffer bees can be trained simultaneously, as well as to ease relocation of sniffer bees from the training to a user friendly portable device during operation.

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