Penerbit Akademia Baru

Journal of Advanced Research Design

Journal homepage:
https://akademiabaru.com/submit/index.php/ard
ISSN: 2289-7984

The Development of Colorimetric Paper-Based Analytical Device for Detection of Sucrose Adulteration in Malaysian Stingless Bee Honey

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ARTICLE INFO

Article history:
Received 10 February 2025
Received in revised form 17 March 2025
Accepted 31 July 2025
Available online 11 August 2025

Keywords:
Stingless bee honey; colorimetric; paper-based analytical device; sucrose; adulteration

ABSTRACT

The growing demand for honey in the market has resulted in tampering honey with foreign substances and increased production of artificial honey. There are lots of laboratory tests for honey adulteration. However, most of the tests are tedious, costly, and complicated. As a result, alternative approaches for faster and simpler detection of honey adulteration should be developed and conducted regularly. Herein, this research attempts to develop paper-based analytical devices (PADs) as a simple, rapid, and inexpensive method for simultaneously detecting sucrose adulteration in stingless bee honey. To develop the PADs, colorimetric paper-based analytical devices (C-PADs) technology was approached. The C-PADs only used a small amount of sample solution (8 µL) for sucrose analyses. Furthermore, no interfering compound was obtained for C-PADs. The storage stability was further investigated, and after 30 days, the stability decreased by 40% from its initial value. Finally, the sucrose content of eight stingless bee honey samples was measured using the C-PADs and compared with high-performance liquid chromatography (HPLC). The results for C-PADs corroborated well with HPLC as most of the results are not significantly different at the 95% confidence level. Among the eight samples, commercial samples H4, H5, and H8 were positive for honey adulteration. From this research, it can be concluded that the C-PADs have been successfully developed to determine sucrose adulteration in stingless bee honey.

1. Introduction

Approximately more than 400 species of stingless bees are found worldwide, with the number of stingless bee species in Malaysia varying between 17 and 32 species [1-3]. According to the Malaysian Agriculture Research and Development Institute (MARDI), the most commercialized stingless bees in Malaysia are *Geniotrigona thoracica* and *Heterotrigona itama*, contributing at least 94.4% in local stingless bee farms [4]. Stingless bees differ from sting bees (*Apis melifera*) in terms of their color, taste, and composition as the differences depend on the types of plants and the nectar that the bee consumes as well as the weather [5,6]. Due to the honey benefits, the demand for it increased and

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https://doi.org/10.37934/ard.143.1.157169

has led to the dishonest act of honey adulteration and the production of synthetic honey. Honey adulteration is an immoral act of producers adding foreign substances such as water, sugar syrups and starch to natural honey [7]. Honey adulteration does not have any serious health issues but it negatively influences the market growth and consumer trust [8].

Over the years, many laboratory techniques and tests for honey adulteration have been developed to determine the purity of honey samples. Methods such as high-performance liquid chromatography (HPLC), Fourier transform infrared spectroscopy, and Raman spectroscopy are commonly used to detect adulteration in honey. However, not all tests are suitable for consumers as testing honey samples in the laboratory is time-consuming, and must be handled by trained operators [9]. Portable on-site testing devices or point-of-care (PoC) devices can be the best solution to the rapid detection of honey adulteration as they emphasize on-site diagnosis that allows test results to be obtained directly by users [10]. Although PoC technologies are often practiced in clinical diagnostic applications, the demand for these technologies in industrial research is increasing.

PoC devices can be developed using various platforms, such as glass, ceramic, plastic, and paper [11]. One of the most developed PoC devices is paper-based analytical devices (PADs) or paper-based sensors, where the idea of PADs is to carry out the analysis on a small piece of paper. By using paper as the platform for PoC devices, the devices are now known as PADs, which can be built as user-friendly, portable and cost-saving devices that can produce rapid results [10,12]. The most common paper types are filter paper, graphite paper and chromatography paper [10,13].

With the advancement of PADs, various detection techniques have been widely applied to quickly quantify the fabricated PADs, such as electrochemical detection, colorimetry assay, transmittance, chemiluminescence, fluorescence, mass spectrum, and surface-enhanced Raman spectroscopy [10,14,15]. Colorimetry is the most frequent detection method associated with PADs, in which particular reagents are applied to the devices and the color intensity produced corresponds to analyte concentration [16]. Colorimetry detection employs relatively simple instrumentation and offers notable benefits, such as visual readout, rapid detection efficiency, remote area applications, straightforward operation, and excellent stability [17].

There are limited studies on PADs that used honey as samples in the past. Most works on PADs reported the detection of sugars (glucose, fructose and sucrose) in wine, fruits and commercial sugary beverages. However, most of these studies involved complicated and costly modification and fabrication techniques. Hence, this research aimed to develop colorimetric PADs that are effective, simple, and affordable in detecting sucrose adulteration in stingless bee honey.

2. Methodology

2.1 Sample Collection

Eight honey samples from different regions in Malaysia were collected. Three pure stingless bee honey samples of *H. itama* and *G. thoracica* were harvested from Universiti Malaysia Pahang stingless bee farm, Kuantan, and Aqif Kelulut Farm, Pekan, while the other five samples were commercially obtained from a local market in Malaysia. All honey samples were stored at 4°C and left at room temperature before the analyses.

2.2 Materials, Chemicals and Reagents

All of the chemicals and reagents used for this research are analytical grade. Sugar standard (Sucrose), acetonitrile, potassium hydrogen phosphate (KH_2PO_4), di-potassium phosphate ($K_2HPO_4.2H_2O$), sodium chloride (NaCl), hydrochloric acid (HCl), acetic acid, sodium acetate, sodium

hydroxide (NaOH), phenol, enzymes Invertase (INV) from baker's yeast (S.cerevisiae), Grade VII, >300 units/mg solid, Glucose Oxidase (GOx) from Aspergillus niger,Type VII, lyophilized powder, ≥100,000 units/g solid (without added oxygen), 10KU were purchased from Sigma-Aldrich (USA), enzyme Horseradish peroxidase (HRP), 145.7 U/mg O-dianisidine dihydrochloride and 4- aminoantipyrine are from Aladdin chemicals (China). Whatman Filter Paper Grade 1 (90mm Ø, Pore size: 11 µm) was obtained from Tay Scientific (Malaysia).

2.3 Fabrication of C-PADs

The C-PADs fabrication method was done following the methods from various literature with some modifications [18,19]. First, 5-mm circle spots were drawn using Microsoft Word before being printed on filter paper using HP Deskjet Ink Advantage 2020hc (Hewlett-Packard, USA). Then, the spot was traced with a Sharpie® permanent marker and left to dry for 5–10 min. The overall fabrication process for C-PADs is illustrated in Figure 1.

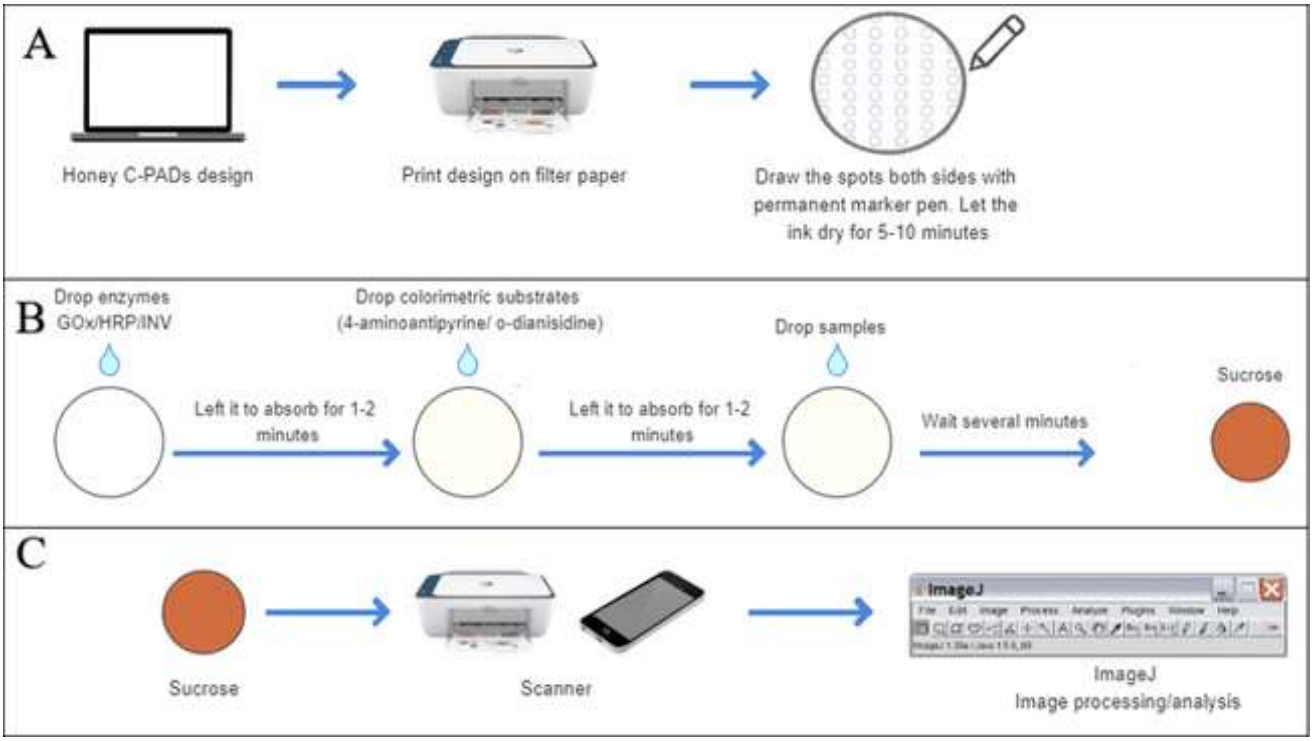

Fig. 1. Overall development of Honey C-PADs. **A**. Fabrication of Honey C-PADs; **B**. Application of samples on developed Honey C-PADs; **C.** Sample analysis using ImageJ software

2.4 Enzymes and Spot Test Preparation

For sucrose spot tests, 100 U/mL stocks of INV and GOx were prepared in acetate buffer, while HRP was prepared in phosphate buffer. For sucrose analysis, 1.5 µL of 80 U/mL of GOx, INV and HRP enzymes and 1.5 µL of 5 mM o-dianisidine dihydrochloride were added to the sample spot. Next, the spots were dried for 1–2 min before 1.5 µL of standard solution (sucrose) or sample was added.

2.5 Application of Samples for Spot Test

For standard sugar, 3.42 g of sucrose was weighed. The standard was dissolved in 100 mL of UPW to achieve a 100 mM concentration. For the honey sample, 5 g of honey was weighed and dissolved

in 100 mL of UPW. Before the analysis, the honey sample was diluted 30 times with UPW. The sample analysis was conducted in triplicate.

2.6 Spot Test Imaging

After the sample was dropped, the test spots were left to dry until the color developed. Then, the color PAD was scanned using a desktop scanner (Hewlett-Packard, USA) and iPhone 12 camera (Apple Inc, USA). The captured images were saved as JPEG files, and the mean intensity of the spots was measured using the red-green-blue color system in ImageJ software (The National Institutes of Health, USA). The intensity of sucrose was evaluated using blue channel. Finally, for sucrose determination in honey samples, three different approaches were used: calibration curves, subtracting negative control from positive control and visual categorization [19].

2.7 Sucrose Detection by HPLC

The sucrose in honey was determined using HPLC 1260 Infinity II LC System (Agilent Technologies, USA) under the following conditions: Zorbax column (Zorbax NH2 150 × 4.6 mm, Agilent Technologies, USA), a column temperature of 35 °C; mobile phase of 75% acetonitrile:25% water; and a flow rate of 0.8 mL/mL. The detection was performed using an RID refractive index detector.

2.8 Statistical Analyses

All the analyses were conducted in triplicate, where the differences between mean values were significant at values of $p < 0.05$. The data obtained in the study were statistically analyzed using a one-way analysis of variance (ANOVA), followed by the Tukey test (Minitab 20.2, Minitab Inc, USA). Besides, an independent t-test also was used to evaluate the difference between C-PADs with HPLC (IBM SPSS Statistic 25.0, IBM, USA).

3. Results

3.1 The Red-Blue-Green Profile of Glucose and Sucrose

Based on Figure 2, the blue channel showed the best signal for sucrose detection. Aksorn & Teepoo [19] also reported similar observations in their study. Hence, for the rest of the experiments, the blue channels were selected for the quantitative analysis of and sucrose.

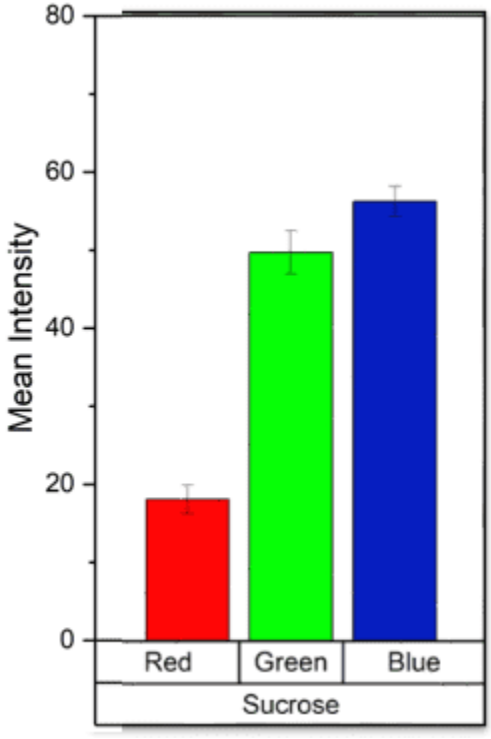

Fig. 2. The Red-Green-Blue colour profile of sucrose C-PADs

3.2 Operating Conditions of C-PADs

The concentration of enzymes was varied from 10 to 100 U/mL and as illustrated in Figure 3, the intensity was maximum at a concentration of 80 U/m and the intensity decreased slightly above 80 U/mL. Thus, a concentration of 80 U/mL was chosen for the detection of sucrose in C-PADs.

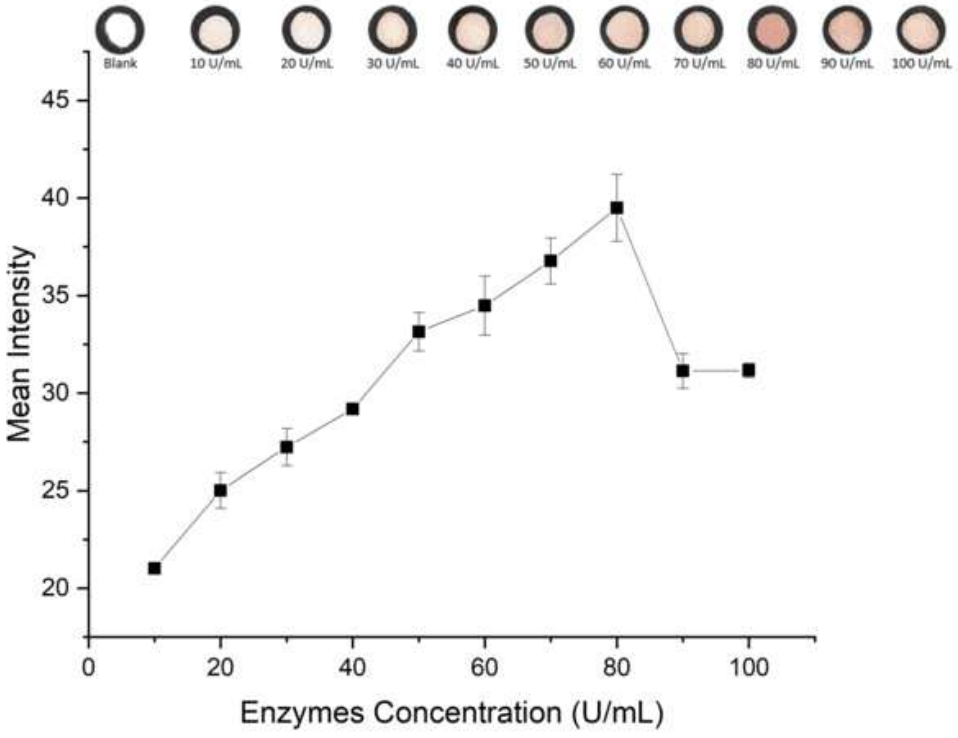

Fig. 3. Enzymes concentration (INV, GOx and HRP) for sucrose reaction in C-PAD

The concentration of o-dianisidine for sucrose reaction was explored where the intensity is higher with the increasing concentration of o-dianisidine (1.0 to 5mM). For detecting sucrose, the sucrose must first be hydrolyzed with invertase, releasing glucose and fructose. The glucose is then detected with the GOD-peroxidase-o-dianisidine system. According to Aksorn and Teepoo [19], the intensity is increasing due to the increasing of o-dianisidone$_{oxi}$ products. However, at a certain concentration level, the intensity appeared to be saturated. The finding was similar to this research where the intensity value remains steady at a concentration greater than 5 mM. Nonetheless, from Figure 4. a concentration of 4 mM was selected to complete the sucrose reaction as it has the highest intensity.

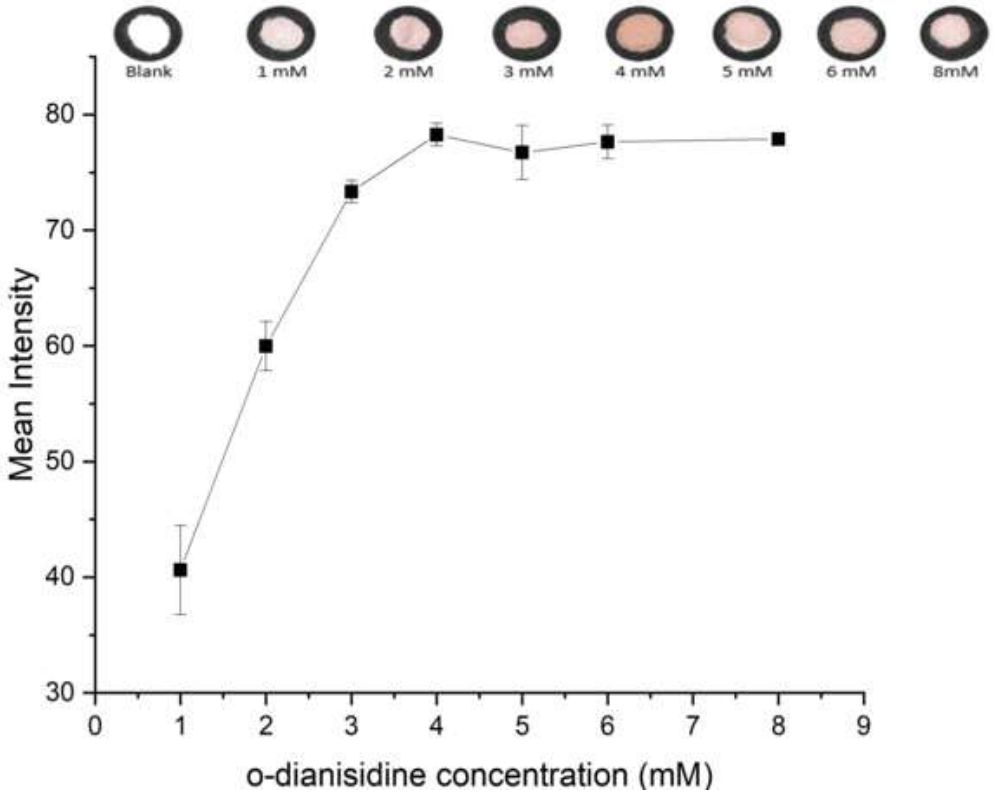

Fig. 4. The variation of o-dianisidine concentration for sucrose detection in C-PAD

By applying the best condition from parameters above, the reaction time for sucrose detection were studied in the range of 2 to 30 min and it took 12 minutes to complete the reaction. This can be seen in Figure 5 where the intensity was at highest at 12 min and kept declining with increasing time.

3.3 Analytical Performance of C-PADs

The analytical performance for sucrose C-PADs was determined including the development of calibration curve, selectivity and reproducibility and storage ability. The conditions used to analyse the analytical performance of C-PADs are tabulated in Table 1.

Table 1
Operating condition for sucrose paper-based analytical devices (C-PADs)

Parameter	Enzymes (U/mL)	Substrates	Reaction time (min)
Sucrose	80 U/mL	4 mM of O-dianisidine	12 min

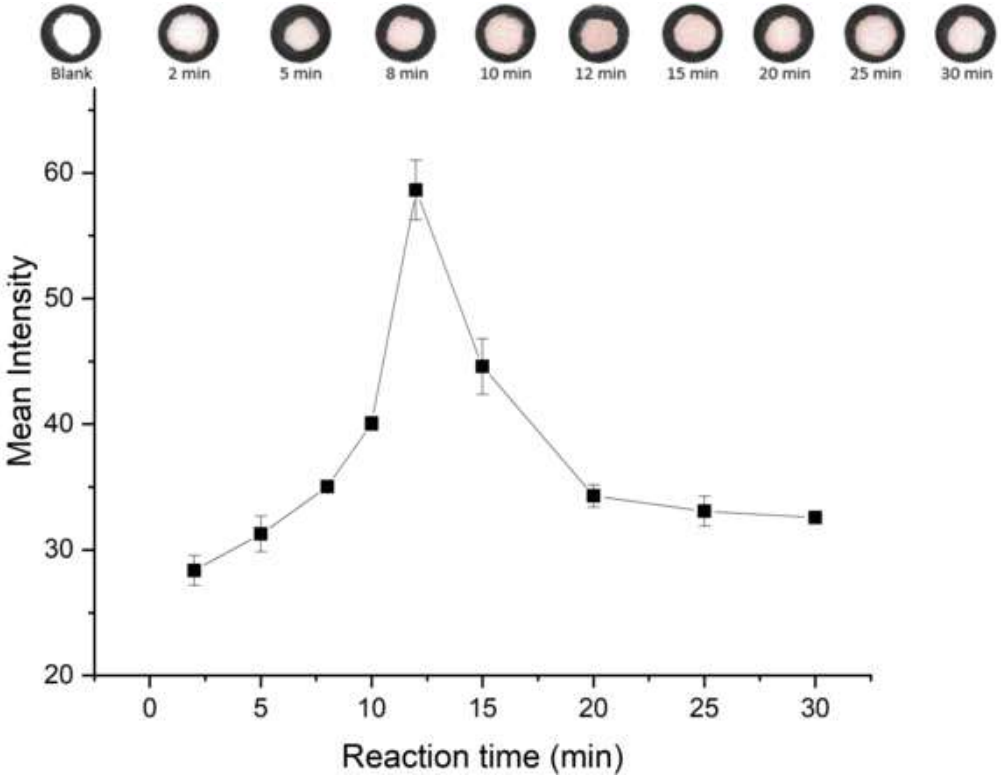

Fig. 5. Reaction time for sucrose detection in C-PAD

3.4 Calibration of C-PADs

Figure 6 showed the sucrose C-PADs had a linear range between 0.5 mM to 12.5 Mm and the calculated LOD is 0.97 mM.

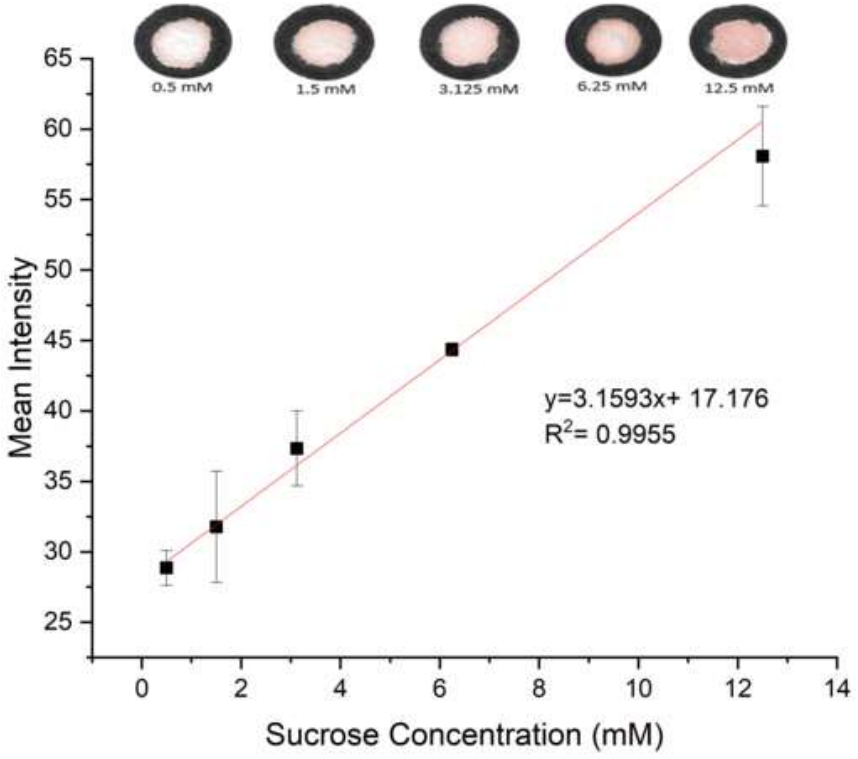

Fig. 6. Calibration curve with various sucrose concentrations for sucrose C-PADs

3.5 The Selectivity of the C-PADs

The selectivity of sucrose C-PADs against the possible interference was investigated. Component such as fructose, lactose, xylose and maltose and ascorbic acid was dropped on the sample test where all of test concentration was fixed at 10 mM. From Figure 7, all of the interfering compounds do not displayed any changes in color and intensity while sucrose has higher intensity. This may be due to the enzyme specificity (invertase and glucose oxidase) towards the reactions involving sucrose [20]. As a result, the developed sucrose C-PADS has high sensitivity for sucrose detection in honey samples.

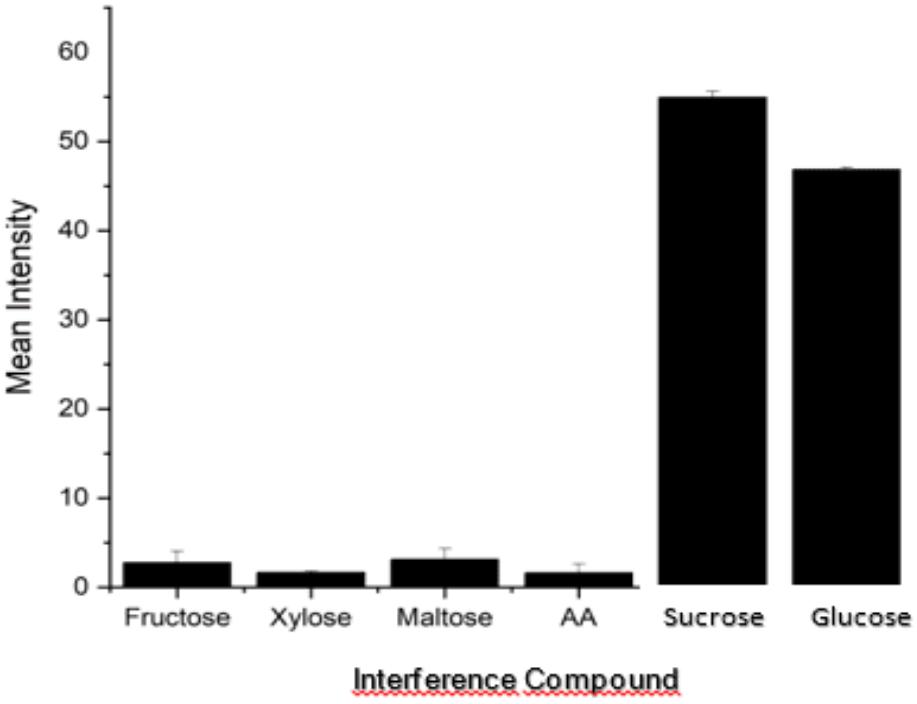

Fig. 7. Selectivity comparison of sucrose with various interferences compound; Fructose, Xylose, Maltose, glucose and AA (Ascorbic acid)

3.6 Repeatability and Precision

For this section, 6 replicates sucrose C-PADs were developed to evaluate the reproducibility. For sucrose C-PADs is 4.36 %. Since the results are lower than 5 %RSD, the sucrose C-PADs had a good precision. Besides replicating the C-PADs, the reproducibility using different instrument (HP Deskjet Ink Advantage 2020hc, Canon E470 Compact Wireless All-In-One and Iphone 12 camera). The C-PADs showing high %RSD (37.1 - 45.8) when measured using a scanner and smartphone camera. The high %RSD may be due to the colour and intensity interpretation variation for different instrument as well as the external/internal light condition [14]. In addition, the accuracy of the C-PADs was also determined using a recovery test where 3 different concentrations (3 mM, 6 mM and 20 mM) of standard solution (sucrose) were spiked into the blank sample. For this purpose, pure honey samples (*Heterotrigona Itama*) were used as blank sample [21]. From Table 2, the recovery ranges were 95.8% to 102.1 % for sucrose. The % recovery obtained was similar to the results by Aksorn and Teepo [19]. According to Artigues *et al.,* [22], the recoveries of the prepared samples should be comprised between 95% and 105% where the value is proposed by the Association of Analytical Communities (AOAC). Therefore, based on the results, the Honey C-PADs were capable to determine sucrose with an acceptable level of accuracy.

Table 2
The recovery test in the spiked stingless bee honey (*Heterotrigona Itama*) sample using glucose and sucrose C-PADs

Analytes	Initial concentration (mM)	Addition (mM)	Total found (mM)	Recovery (%)
Sucrose	1.31 ± 0.29	5	6.20 ± 0.67	97.7
Sucrose	1.95 ± 0.29	8	10.0 ± 0.20	101.0
Sucrose	1.95 ± 0.29	12	14.2 ± 0.41	102.1

3.7 Stability of C-PADs

Figure 8 shows that the intensity of sucrose decreased to 40% and 37.9% of its initial value after 4 weeks' storage in 4°C and -20 °C, respectively. There is no significant difference was observed for both storage temperatures, thus it was advisable to store the C-PADs in cold temperature for best activity.

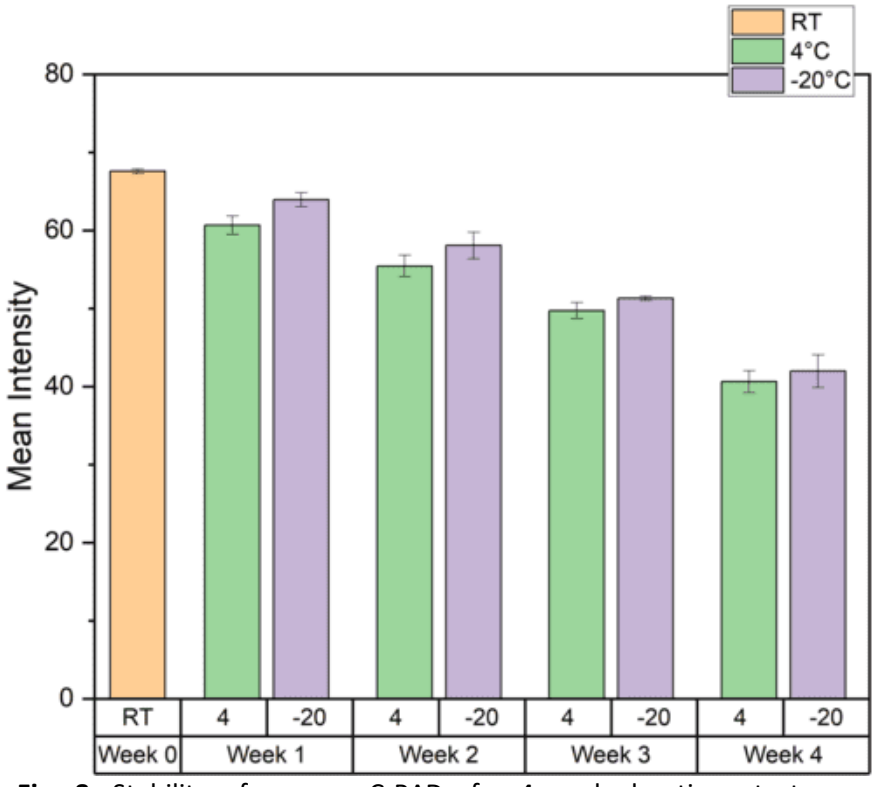

Fig. 8. Stability of sucrose C-PADs for 4-week duration at storage temperature of 4°C and – 20 °C

3.8 Application of C-PADs on Honey Samples

The performance of Honey C-PADs was further investigated by analysing their detection ability in eight honey samples. The obtained results were then statistically compared with HPLC method. In Table 3, based on independent t-test, there were no significant differences ($p > 0.05$) of sucrose level between C-PADs and HPLC methods for all samples except for H1 ($p=<0.001$) and H2 ($p=0.003$). The difference was expected since the lowest sucrose concentration that C- PADs able to detect was at 0.97 mM while HPLC can detect as low as 0.23 mM (LOD).

Table 3
The measurement of sucrose between C-PADs and HPLC methods

Samples	Sucrose C-PADs (g/100g) Mean±s.d.	HPLC (g/100g) Mean±s.d.	Mean diff. (95% CI)	p-value
H1	1.10 ± 0.10	0.39 ± 0.02	-0.715 (-0.873,-0.556)	< 0.001*
H2	1.24 ± 0.09	0.87 ± 0.08	-0.363 (-0.517,-0.210)	0.003*
H3	1.14 ± 0.10	0.95 ± 0.06	-0.191 (-0.396,0.136)	0.061
H4	14.8 ± 0.79	14.1 ± 0.02	0.683 (-1.954,0.587)	0.210
H5	22.2 ± 0.48	22.9 ± 0.10	0.766 (-0.046,1.580)	0.059
H6	3.35 ± 0.23	2.97 ± 0.01	-0.384(-0.782,0.135)	0.055
H7	1.19 ± 0.13	1.02 ± 0.04	0.175 (-0.417,0.067)	0.116
H8	21.9 ± 0.15	22.3 ± 0.07	0.312 (-0.710,1.333)	0.444

Note: *Significantly different if p-value <0.05

As for positive control evaluation, the calculated positive internal limit was at 61.3. From all samples, H4, H5 and H8 ranged from 115.12 to 155.95 passed the control limit, hence, the samples may be adulterated with sucrose/cane sugar (Figure 9). To classify the 8 honey samples visually, the obtained colour of each samples was compared to the negative (light brown) and positive (brown) controls simultaneously. If the sample was browner than the positive control, it was considered adulterated. Based on Figure 10, H4, H5 and H8 are darker in colour when compared to positive control, thus, it conforms that these three honey may be adulterated with foreign substances.

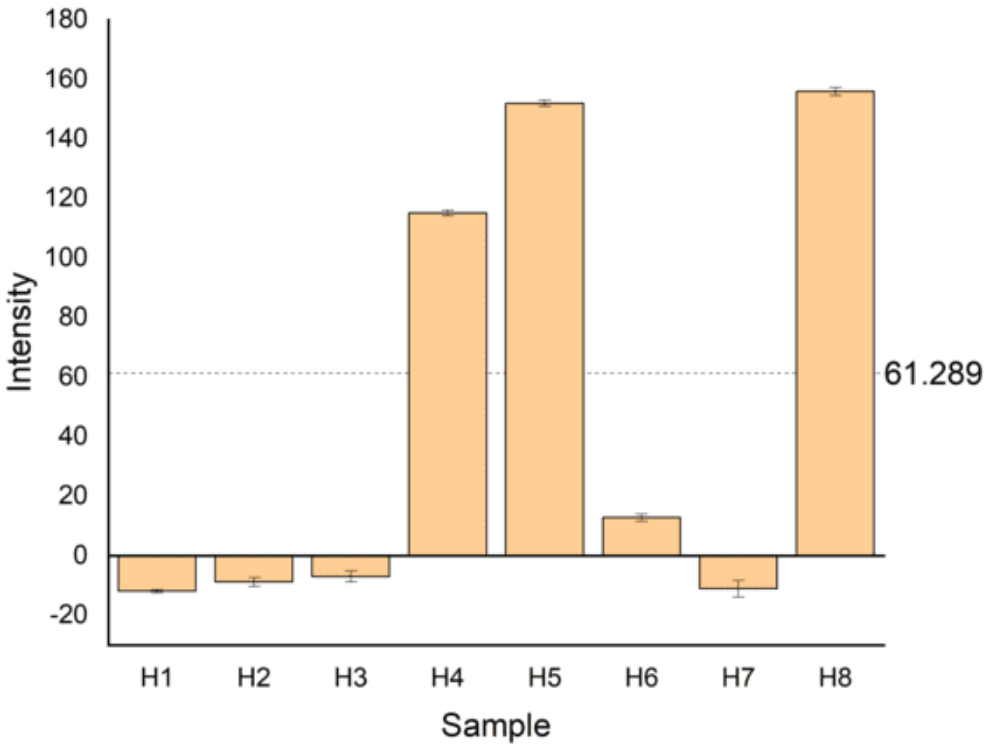

Fig. 9. Sucrose intensity determination of 8 stingless bee honey using positive control evaluation

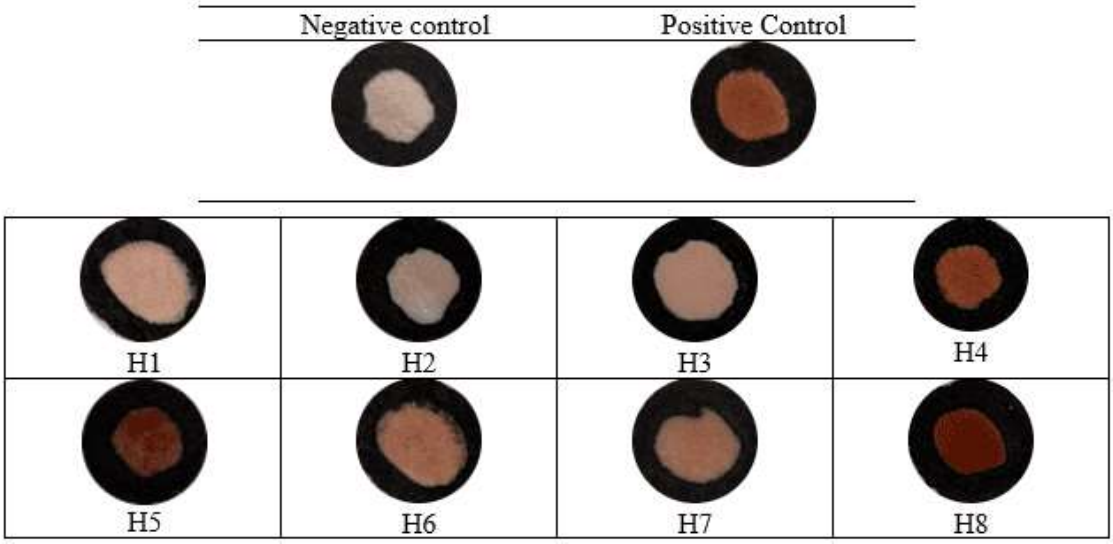

Fig. 10. The color intensity of negative control, positive internal control and 8 stingless bee honey samples of sucrose C-PADs

4. Conclusions

In conclusion, a low-cost paper-based analytical device (PADs) for determining sucrose adulteration in stingless bee honey samples has been developed based on colorimetric detection. Overall, the C-PADs successfully detected sucrose analytes in stingless bee honey where the process was quick, simple to fabricate and cost-effective. With a small volume of samples, enzymes, and reagents (1 to 8 µL) and reaction time of 12 minute, the PADs are economical as it saves time and excessive usage of expensive chemical. Furthermore, the sample's result corroborates well with the HPLC method as most samples were not significantly different based on the independent t-test ($p>0.05$). Finally, the C-PADs has more potential to be commercialized in future as the procedure is simpler, rapid and cheaper. The total cost for the materials including the paper devices, enzymes, substrates and buffer is predicted to be roughly RM 8 per device. Nevertheless, both PADs has promising prospects for applications in the detection of glucose and sucrose adulteration in stingless bee honey.

Acknowledgement

The authors would like to thank the Tabung Persidangan dalam Negara (TPDN), Universiti Malaysia Pahang Al-Sultan Abdullah and was supported by the Ministry of Education through Mygrants (Ref: FRGS/1/2019/STG05/UMP/02/7; RDU 1901187) and University Malaysia Pahang Al-Sultan Abdullah (Ref: RDU 1803170, RDU233010).

References

[1] Aidoo, Kwame, Rofela Combey Kwapong, and I. Afia Karikari. "Stingless bees in Ghana." *Bees for Development Journal* 100 (2011): 10-11.

[2] Mustafa, Mohd Zulkifli, Nik Soriani Yaacob, and Siti Amrah Sulaiman. "Reinventing the honey industry: Opportunities of the stingless bee." *The Malaysian journal of medical sciences: MJMS* 25, no. 4 (2018): 1. https://doi.org/10.21315/mjms2018.25.4.1

[3] Salim, Hannah MW, Ahmad Dzamir Dzulkiply, Rhett D. Harrison, Christine Fletcher, Abd Rahman Kassim, and Matthew D. Potts. "Stingless bee (Hymenoptera: Apidae: Meliponini) diversity in dipterocarp forest reserves in Peninsular Malaysia." *Raffles Bulletin of Zoology* 60, no. 1 (2012). Retrieved from https://lkcnhm.nus.edu.sg/wp-content/uploads/sites/10/app/uploads/2017/06/60rbz213-219.pdf

[4] Kelly, N., M. S. N. Farisya, T. K. Kumara, and P. Marcela. "Species Diversity and External Nest Characteristics of Stingless Bees in Meliponiculture." *Pertanika Journal of Tropical Agricultural Science* 37, no. 3 (2014). Retrieved fromhttps://www.researchgate.net/publication/281774057_Species_Diversity_and_External_Nest_Characteristics_of_Stingless_Bees_in_Meliponiculture/link/565d4fe808aeafc2aac732d2/download

[5] Aziz, Muhammad Shakir Abdul, Nelli Giribabu, Pasupuleti Visweswara Rao, and Naguib Salleh. "Pancreatoprotective effects of Geniotrigona thoracica stingless bee honey in streptozotocin-nicotinamide-induced male diabetic rats." *Biomedicine & pharmacotherapy* 89 (2017): 135-145.. https://doi.org/10.1016/j.biopha.2017.02.026

[6] Pasupuleti, Visweswara Rao, Lakhsmi Sammugam, Nagesvari Ramesh, and Siew Hua Gan. "Honey, propolis, and royal jelly: a comprehensive review of their biological actions and health benefits." *Oxidative medicine and cellular longevity* 2017 (2017).https://doi.org/10.1155/2017/1259510.

[7] Islam, Md Nazmul, Md Ibrahim Khalil, Md Asiful Islam, and Siew Hua Gan. "Toxic compounds in honey." *Journal of Applied Toxicology* 34, no. 7 (2014): 733-742.. https://doi.org/10.1002/jat.2952.

[8] J. Head, J. Kinyanjui, M. Talbott, FTIR-ATR Characterization of Commercial Honey Samples and Their Adulteration with Sugar Syrups Using Chemometric Analysis Pittcon 2015, (2015) 1–5. Retrieved from https://www.shimadzu.co.kr/sites/shimadzu.co.kr/files/pim/pim_document_file/technical/white_papers/10512/sia215007.pdf

[9] Z. Gan, Y. Yang, J. Li, X. Wen, M. Zhu, Y. Jiang, Y. Ni, Using sensor and spectral analysis to classify botanical origin and determine adulteration of raw honey, J. Food Eng. 178 (2015) 151–158. https://doi.org/10.1016/j.jfoodeng.2016.01.016.

[10] Liu, Shuopeng, Wenqiong Su, and Xianting Ding. "A review on microfluidic paper-based analytical devices for glucose detection." *Sensors* 16, no. 12 (2016): 2086.https://doi.org/10.3390/s16122086.

[11] Chen, Xi, Jin Chen, Fubin Wang, Xia Xiang, Ming Luo, Xinghu Ji, and Zhike He. "Determination of glucose and uric acid with bienzyme colorimetry on microfluidic paper-based analysis devices." *Biosensors and Bioelectronics* 35, no. 1 (2012): 363-368. https://doi.org/10.1016/j.bios.2012.03.018.

[12] Fu, Lung-Ming, and Yao-Nan Wang. "Detection methods and applications of microfluidic paper-based analytical devices." *TrAC Trends in Analytical Chemistry* 107 (2018): 196-211.L.https://doi.org/10.1016/j.trac.2018.08.018.

[13] Songjaroen, Temsiri, Wijitar Dungchai, Orawon Chailapakul, and Wanida Laiwattanapaisal. "Novel, simple and low-cost alternative method for fabrication of paper-based microfluidics by wax dipping." *Talanta* 85, no. 5 (2011): 2587-2593. https://doi.org/10.1016/j.talanta.2011.08.024.

[14] Liana, Devi D., Burkhard Raguse, J. Justin Gooding, and Edith Chow. "Recent advances in paper-based sensors." *sensors* 12, no. 9 (2012): 11505-11526. https://doi.org/10.3390/s120911505.

[15] V. Bee, C.L. Ee, N. Faizah, M.O.H.D. Aim, E.T. Ami Lee, Vivian Bee Chin, Noor Faizah Mohd-Naim, Eiichi Tamiya, and Minhaz Uddin Ahmed. "Trends in paper-based electrochemical biosensors: from design to application." Analytical Sciences 34, no. 1 (2018): 7-18.https://doi.org/10.2116/analsci.34.7

[16] Kong, Fen-Ying, Sai-Xi Gu, Wei-Wei Li, Ting-Ting Chen, Qin Xu, and Wei Wang. "A paper disk equipped with graphene/polyaniline/Au nanoparticles/glucose oxidase biocomposite modified screen-printed electrode: Toward whole blood glucose determination." Biosensors and Bioelectronics 56 (2014): 77-82.. 56 (2014) 77–82. https://doi.org/10.1016/j.bios.2013.12.067.

[17] Rattanarat, Poomrat, Wijitar Dungchai, David Cate, John Volckens, Orawon Chailapakul, and Charles S. Henry. "Multilayer paper-based device for colorimetric and electrochemical quantification of metals." *Analytical chemistry* 86, no. 7 (2014): 3555-3562.https://doi.org/10.1021/ac5000224.

[18] Fernandes, Gabriel Martins, Weida R. Silva, Diandra Nunes Barreto, Rafaela S. Lamarca, Paulo Clairmont F. Lima Gomes, João Flávio da S Petruci, and Alex D. Batista. "Novel approaches for colorimetric measurements in analytical chemistry–A review." *Analytica Chimica Acta* 1135 (2020): 187-203.. https://doi.org/10.1016/j.aca.2020.07.030.

[19] J Aksorn, Jinakan, and Siriwan Teepoo. "Development of the simultaneous colorimetric enzymatic detection of sucrose, fructose and glucose using a microfluidic paper-based analytical device." *Talanta* 207 (2020): 120302. https://doi.org/10.1016/j.talanta.2019.120302.

[20] Oyewunmi, Oyejide Damilola, Seyed Hamid Safiabadi-Tali, and Sana Jahanshahi-Anbuhi. "Dual-modal assay kit for the qualitative and quantitative determination of the total water hardness using a permanent marker fabricated microfluidic paper-based analytical device." *Chemosensors* 8, no. 4 (2020): 97. https://doi.org/10.3390/chemosensors8040097

[21] Mani, Naresh Kumar, Anusha Prabhu, Sujay Kumar Biswas, and Suman Chakraborty. "Fabricating paper based devices using correction pens." *Scientific Reports* 9, no. 1 (2019): 1752. https://doi.org/10.1038/s41598-018-38308-6.

[22] Artigues, Margalida, Jordi Abellà, and Sergi Colominas. "Analytical parameters of an amperometric glucose biosensor for fast analysis in food samples." *Sensors* 17, no. 11 (2017): 2620. https://doi.org/10.3390/s17112620.