Malaysian Journal of Analytical Sciences (MJAS)
Published by Malaysian Analytical Sciences Society

MODIFIED CHRYSIN-BASED BIOSORBENT FOR THE DISPERSIVE MICRO-SOLID PHASE EXTRACTION OF SELECTED PHENOLIC ACIDS FROM STINGLESS BEE HONEY

(Biopenjerap yang Diubahsuai berasaskan Krisin untuk Pengekstrakan Fasa Pepejal-mikro Asid Fenolik Terpilih secara Serakan daripada Madu Lebah Tanpa Sengat)

NyukTing Ng[1], Aemi Syazwani Abdul Keyon[1,2*], Wan Aini Wan Ibrahim[1*], Mohd Marsin Sanagi[1] and Faridah Mohd Marsin[3]

[1]*Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia*
[2]*Centre for Sustainable Nanomaterials, Ibnu Sina Institute for Scientific and Industrial Research, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia*
[3]*Jabatan Kimia Malaysia Negeri Pahang, Jalan Dato' Bahaman, Alor Akar, 25662 Kuantan, Pahang, Malaysia*

[*]*Corresponding author: aemi@utm.my; waini@utm.my*

Received: 13 September 2023; Accepted: 30 December 2023; Published: 28 February 2024

Abstract

This study focuses on the preparation, characterisation, and application of a chrysin-based biosorbent (TA-CHY) for extracting protocatechuic acid (PCA) and vanillic acid (VA) from stingless bee honey (SBH) using a dispersive micro-solid phase extraction method. The extracted analytes were separated using high-performance liquid chromatography and detected using an ultraviolet detector. TA-CHY was prepared by depositing tannic acid onto the amino-functionalized chrysin. Its point of zero charge was determined at pH 6.8. Fourier transform infrared spectroscopy analysis had identified the presence of C=N, C=C, O-H, SiOR and C-O groups in TA-CHY, while aggregate formation was observed on TA-CHY through scanning electron microscopy. Energy dispersive X-ray study revealed the elemental composition of TA-CHY was 52.9% of C, 34.0% of O, 6.80% of Si and 6.3% of N. Its specific surface area was found to be 5.913 m^2/g via Brunauer-Emmett-Teller surface area analysis. Optimised extraction parameters were 1 min extraction time, 300 µL methanol as desorption solvent, 15 min desorption time, sample pH of 2, no NaCl salt addition, 0.20 g biosorbent mass and 2 mL ethanol as dispersant solvent. The developed method was validated using matrix-match calibration where real SBH samples spiked with PCA and VA in the concentration range of 0.5-50 mg/L. The method exhibited satisfactory analytical performance characteristics, with a limit of detection ranging from 0.81 to 1.37 mg/kg, a limit of quantification ranging from 2.69 to 4.56 mg/kg, relative recoveries ranging from 79.38% to 113.38%, and precision with relative standard deviation values ranging from 0.29% to 10.83%. Importantly, the proposed method obtained a score of 71 on the analytical Eco-Scale, indicating its acceptability as a green method.

Keywords: chrysin, biosorbent, dispersive micro-solid phase extraction, phenolic acids, stingless bee honey

Abstrak

Kajian ini memberi tumpuan pada penyediaan, pencirian, dan penggunaan biopenjerap berasaskan krisin (TA-CHY) untuk mengekstrak asid protokatekuik (PCA) dan asid vanillik (VA) daripada madu lebah (SBH) tanpa sengat dengan menggunakan

kaedah pengekstrakan fasa pepejal-mikro secara serakan. Analit yang diekstrak telah diasingkan menggunakan kromatografi cecair berprestasi tinggi dan dikesan menggunakan pengesan ultraungu. Biopenjerap TA-CHY telah disediakan dengan mendepositkan asid tannik pada krisin yang telah diberikan fungsi amino. Cas sifar titiknya telah ditentukan pada pH 6.8. Analisis spektroskopi inframerah transformasi Fourier telah mengenalpasti kehadiran kumpulan C=N, C=C, O-H, SiOR dan C-O dalam TA-CHY manakala pembentukan agregat diperhatikan pada TA-CHY melalui mikroskop pengimbas electron. Kajian tenaga penyerakan sinar-X mendedahkan komposisi unsur bagi TA-CHY ialah 52.9% C, 34.0% O, 6.80% Si dan 6.3% N. Luas permukaan spesifiknya didapati sebanyak 5.913 m^2/g melalui analisis kawasan permukaan Brunauer-Emmett-Teller. Parameter pengekstrakan yang dioptimumkan ialah 1 minit masa pengekstrakan, 300 µL metanol sebagai pelarut desorpsi, 15 minit masa desorpsi, pH sampel 2, tiada penambahan garam NaCl, 0.20 g jisim biopenjerap dan 2 mL etanol sebagai pelarut penyerakan. Kaedah yang dibangunkan telah disahkan menggunakan kalibrasi padanan-matriks dengan sampel SBH tulen dengan menambah PCA dan VA dalam lingkungan kepekatan 0.5-50 mg/L. Kaedah ini menunjukkan ciri prestasi analisis yang memuaskan, dengan had pengesanan antara 0.81 dan 1.37 mg/kg, had kuantitatif antara 2.69 dan 4.56 mg/kg, pemulihan relatif antara 79.38% dan 113.38%, dan ketepatan dengan sisihan piawai relatif, antara 0.29% dan 10.83%. Kaedah yang dicadangkan telah memperoleh markah 71 pada skala-Eko analitik yang menunjukkan kebolehterimaannya sebagai kaedah hijau adalah sangat penting.

Kata kunci: krisin, biopenjerap, pengekstrakan fasa pepejal-mikro secara serakan, asid fenolik, madu lebah tanpa sengat

Introduction

Stingless bee honey (SBH) is a remarkable natural sweetener that possesses several unique physicochemical characteristics, namely high acidity (pH 3.07-3.19), high moisture content (20.00-33.24 g/100 g honey), and low sugar content (50.35-62.38 g/100 g honey) [1, 2]. It is well-known for its unique formulation of bioactive compounds, notably phenolic acids (PAs). PAs are bioactive compounds with potent anti-inflammatory and anti-oxidant properties [3, 4], making their analysis in honey crucial for assessing honey quality and potential health-promoting effects. However, the complicated matrix of the viscous and hygroscopic honey sample makes it difficult to determine the presence of PAs in a direct way. This challenge inspired researchers and thereby promoted the development of more effective and specialized sample preparation methods for SBH [5]. As the search for eco-friendly and sustainable analytical methods has gained prominence, and green analytical chemistry ideas are now more widely acknowledged [6]. It is critical to develop efficient, innovative and environmentally friendly sample preparation procedures for assessing PAs in honey.

Sample preparation methods, such as solvent extraction (SE), liquid-liquid extraction (LLE) and solid-phase extraction (SPE), have been commonly employed for PA analysis [7]. These methods are relatively cheap and simple, without the need of expensive, complicated and specialized instrumentation. Nonetheless, these methods are often associated with drawbacks, including the use of large volumes of organic solvents, which are not environmentally friendly and may pose potential health hazards. For instance, the main disadvantages of LLE are the high volume of solvents used (from a few mL to L of ethyl acetate and diethyl ether) and the emulsion formation, which makes it challenging to recover some phenolic compounds effectively. To overcome emulsion problem, addition of surfactant (methanol and ethanol), the need of ultrasonication, freezing out after separation of phases and gentle sample shaking might be needed. While these solutions are less favorable as addition of surfactant may affect the partition equilibrium between phases, additional steps like shaking, ultrasonication and freezing out could be laborious and may need a longer time to perform extraction. More organic solvent also may be needed for rinsing after the freezing out process.

In SPE, commercial SPE cartridges often require smaller quantities of samples and low amounts of organic solvents while its vacuum manifold and vacuum pumps might be costly for the analysis of a reduced number of samples. The number of stages should be kept to a minimum because each stage or transfer might result in loss of analytes. These limitations motivated analytical chemists to improve and develop new mode of SPE methods and one of these methods is dispersive-micro-solid phase extraction (d-µ-SPE). Furthermore, various analytical approaches have been used to analyze PAs, each with its own set of limitations. Thin-layer chromatography, which employs silica gel, cellulose, or polyamide layers, provides a quick and cost-effective separation process. However, its disadvantage is that it has limited quantitation capabilities due to the loss of analytes during sample processing. The Folin-Ciocalteu reagent, which has traditionally been used for colorimetric measurement of total phenolic content has difficulty in identifying individual phenolic components. This is mostly due to the involvement of other components in food extracts that function as reducing agents. However, employing gas chromatography for

the separation of PAs is challenging as these phenolic compounds have low volatility and derivatisation is necessary. Reversed phase high-performance liquid chromatography (HPLC) provides effective separation of PAs, nevertheless, there were limited studies that focused on PAs. Hence, HPLC was employed for the separation of PAs in this study.

The first d-μ-SPE was developed by Anastassiades et al. [8]. It is one of the desirable methods for sample preparation due to its capacity to offer improved sensitivity, adaptability, and compatibility with automation [9]. When compared to SE, LLE and SPE, d-μ-SPE usually uses far less organic solvent (few μL to mL), making it a greener and a more economical option. It also tends to be quicker than SE and LLE as only few minutes needed for its sorption-desorption process [10]. Besides that, d-μ-SPE may give high selectivity for target analytes by using the right sorbent and experimental conditions. Therefore, sorbent is a core element of this method, facilitating the efficient separation of analytes from complicated matrices. Dispersive mode of SPE typically employs sorbent materials based on silica, layered double hydroxides, magnetic nanomaterials, porous polymers, metal organic framework, and even carbonaceous materials such as activated carbon, graphene, graphite and carbon nanotubes [11]. Furthermore, the development of biosorbents has become imperative in order to make full use of natural resources which includes magnetic cork composites [12], seed [13], tannic acid [14] and many others. Careful sorbent selection is significant in obtaining optimum analytical performances. However, ensuring the high efficiency and effectiveness of an analytical method is equally important as assuring its environmental sustainability.

Flavonoids, which are plentiful in nature, provide a sustainable and biocompatible alternative to traditional sorbents [10, 15]. In this study, d-μ-SPE was developed and combined with a novel chrysin-based biosorbent, TA-CHY for the analysis of vanillic acid (VA) and protocatechuic acid (PCA) in SBH. Chrysin, a natural flavonoid present in propolis and honey in large quantities [16] and shares structural similarities with PAs (benzene rings and hydroxyl groups). The modification of chrysin as a biosorbent which offer numerous advantages, including eco-friendliness, simplicity, and high potential to extract PAs. Since the chrysin biosorbent has remarkable affinity and selectivity towards PAs, it enables effective preconcentration of PAs from difficult matrices like SBH. The distinctive structural characteristics, such as its surface morphology and the presence of functional groups (hydroxyl groups, carbonyl groups, benzene ring), play a key role in increasing its ability to bind analytes, resulting in better analyte recovery and sensitivity [17]. The analysis of PAs in SBH is of great importance for assessing its quality. Therefore, this innovative approach is expected to contribute to the advancement of green analytical practices and provides researchers with a reliable method for the analysis of PAs in SBH. The eco-friendliness of the newly developed method was assessed by analytical Eco-scale metric proposed by Gałuszka et al. [18] to ensure the method align with the principles of green analytical chemistry.

Materials and Methods

Chemicals and reagents

Potassium dihydrogen phosphate, sodium nitrate, sodium hydroxide pellets, ortho-phosphoric acid (85%), nitric acid (65%), sodium chloride and tannic acid were supplied by Merck (Darmstadt, Germany). Analytical grade of toluene, *n*-hexane and acetonitrile were purchased from Thermos Fisher Scientific (Massachusetts, USA), Qrec (Selangor, Malaysia) and Qrec (Selangor, Malaysia), respectively. VA (97%) and PCA were obtained from Sigma-Aldrich (St. Louis, MO, USA). Absolute ethanol used in this study was obtained from VWR International S.A.S. (Fontenay-sous-Bois Cedex, France). Chrysin (99+%) and 3-aminopropyltriethoxysilane (APTES, 99%) were obtained from Acros Organics B.V.B.A. (Geel, Belgium). Methanol and isopropanol were provided by J.T. Baker (Center Valley, PA, USA). The ultrapure water (18.2 MΩ) was collected from Milli-Q gradient water system (Millipore, Massachusetts, USA). All analytical grade chemicals were used without further purification. 1000 mg/L stock solution of each analyte (PCA and VA) were separately prepared in methanol. After that, the stock solution of each analyte was utilized to make their respective single standard solutions of 100 and 10 mg/L. A sample solution was always made fresh before use. When not in use, the stock standard solutions were stored at 4 °C.

Instruments and apparatus

Fourier-transform infrared (FTIR) characterisation was carried out utilizing a PerkinElmer FrontierTM FT-IR spectrometer paired with a polarized UATR (Massachusetts, USA). Surface morphology was studied using a Jeol JSM-IT300LV scanning electron microscopy (SEM) from Essex County, Massachusetts, at 10-15 kV, 1000-2000× magnification, and a working

distance of 10.4-11.1 mm. The elemental composition was investigated using an energy dispersive X-ray spectroscopy (EDX) from Oxford Instrument X-max in Oxford, UK. The *Brunauer-Emmett-Teller* (BET) surface areas of the biosorbent were investigated using a NOVAtouch surface area and pore size analyser acquired from Quantachrome Corporation, Boynton Beach, FL, USA. The pH of the solution was measured using a Eutech pH meter, model pH 700 (Paisley, UK). The sorption equilibration of the biosorbent during point of zero charge measurement (pH_{pzc}) was facilitated by the use of a PROTECH orbital shaker (model 722). A ZX4 vortex mixer was purchased from VELP SCIENTIFICA, Italy for extraction purpose. A FAVORIT stirring hotplate (HS0707V2) was used to stir the solution. A KUBOTA 2420 centrifuge machine, made in Tokyo, Japan, was used for liquor separation. A BRANSON CPX3800H ultrasonicator (Danbury, CT, USA) was used to desorb analytes from the biosorbent surface and to degas the buffer solution. Mass of biosorbent was measured by using a Sartorius ENTRIS224-1S analytical balance (Göttingen, Germany).

The VA and PCA analyses were performed using a reversed phase HPLC (model 1220 Infinity LC) combined with a ultraviolet detector (G4288C). The HPLC was equipped with a 5 m, 2.1×150 mm Eclipse Plus C_{18} separating column. Both HPLC instrument and the separating column were purchased from Agilent Technologies, Santa Clara, CA, USA. The wavelength of detection for VA and PCA was fixed at 280 nm. An isocratic mobile phase, buffer-methanol (90:10) (v/v), was utilized for the chromatographic separation. First, 3.4022 g of potassium dihydrogen phosphate was dissolved in ~480 mL of ultrapure water. Next, the buffer pH was carefully adjusted to 3 using ortho-phosphoric acid or/and sodium hydroxide solution, followed by diluting the buffer to desired volume mark (500 mL) for the making of 0.05 M buffer solution. The prepared buffer was filtered using an LC solvent filter assembly (Agilent technologies, Santa Clara, CA, USA) paired with a 47 mm diameter nylon membrane filter (Whatman, Buckinghamshire, UK). The injection flow rate and volume were set at 0.5 mL/min and 20 µL, respectively. ChemStation software was used to process the chromatographic data. The peaks for PCA and VA showed on the chromatogram at ~4.2 and ~13.25 min, respectively.

Preparation of TA-CHY biosorbent

0.8 g of chrysin was weighed and dispersed in 80 mL of ethanol via stirring. Then, APTES (3%, v/v) prepared in 20 mL ethanol was added dropwise to the stirring mixture, followed by the addition of 2 g of tannic acid to the mixture. The mixture was stirred at room temperature for 24 hours. After that, the solid product was separated from the solvent mixture via filtration. It was rinsed with ethanol and deionized water for several times. At last, the solid product was collected and dried for 24 hours at 60°C.

Point of zero charge determination

Salt addition method was used to determine the pH_{pzc} of TA-CHY. First, initial pH value ($pH_{initial}$) of a 0.1M $NaNO_3$ solution (10 mL) was set at 3, adjusted with nitric acid or sodium hydroxide solutions. Then, in a 50 mL conical flask, 0.1 g of TA-CHY was dispersed uniformly in the $NaNO_3$ solution. The mixture was equilibrated for 24 hours using a mechanical orbital shaker at 200 rpm. At last, final pH, pH_{final} of the $NaNO_3$ solution was measured and recorded. The experiment was repeated with different pHi_{nitial} values (pH 5, 7, and 9), their corresponding pH_{final} values were determined as well.

Dispersive-micro solid phase extraction

A 45 mL of sample solution (pH 5) was poured to a centrifuge tube containing 0.15 g of TA-CHY dispersed in 2 mL of dispersant solvent, ethanol. Then, a vortex mixer was used to extract the analytes, PCA and VA from the sample for one min at 900 rpm. After that, TA-CHY was separated via centrifugation. The collected biosorbent was added with 300 µL of methanol as desorption solvent, followed by ultrasonically desorbed for 15 min. The extract was then separated again via centrifugation and dried under nitrogen flow. The dried sample was added with 150 µL of mobile phase (buffer: methanol at 9:1 ratio) as sample solvent. Finally, the reconstituted extract (20 µL) was injected to the HPLC instrument.

Real sample preparation

Four raw samples of SBH were collected from Alor Gajah (Malacca), Batang Kali, Hulu Selangor (Selangor), Mata Air, Chuping (Perlis) and Taiping (Perak) in Malaysia. Next, 10 g of honey was dissolved in 100 mL of ultrapure water to make the sample. Sodium hydroxide or ortho-phosphoric acid were used to adjust the pH of the diluted sample (~95 mL) to 2. Then, the pH-adjusted sample was diluted to the desired volume, 100 mL. The prepared sample was then readied for further study. When not in use, the SBH samples were kept in a dark, dry area at room temperature.

Method validation

The developed d-μ-SPE was verified using matrix-matched calibration by assessing a series of genuine SBH samples spiked with different levels of analytes. Its analytical characteristics, namely linearity, sensitivity in terms of limits of quantification (LOQ, 10(standard deviation of the response, σ/slope of the calibration curve, S)) and limits of detection (LOD, 3(σ/S)), accuracy in terms of relative standard deviation (RSD), and recovery in terms of relative recovery (RR) were investigated. The linearity ranges of both PVA and VA were 0.5-50 mg/L, and their model fitnesses were assessed through the coefficient of determination, R^2. Three replicate samples were examined on the same day (n=3) and different days (n=9) for intra- and inter-day precision investigations. The percentage of the recovered analyte in the spiked sample vs the known analyte in the spiked sample was expressed as the RR in this study.

Results and Discussion

Biosorbent characterization

A multidisciplinary approach was used to study the properties of TA-CHY such as studying pH_{pzc} using salt addition method, molecular compositions using FTIR, surface morphology and elemental analysis using SEM-EDX, and specific surface area using BET which revealed comprehensive insights into the structural and chemical characteristics of TA-CHY.

Surface morphology, elemental composition and surface area study

The SEM images of chrysin, tannic acid, and TA-CHY are shown in Figure 1. Figure 1(a) depicts pristine tannic acid as spherical with a smooth surface. In contrast, pristine chrysin has a cylindrical form with a rough surface, as seen in Figure 1(b). Figure 1(c) depicts SEM images of TA-CHY with some aggregate formation on the surface of tannic acid, demonstrating that the deposition of chrysin on the tannic acid surface was effective with the aid of APTES. Additional confirmation was obtained in the EDX results (Table 1), where the weight percentages of components changed when compared to the pristine chrysin and tannic acid. The presence of Si and N components in TA-CHY demonstrated that APTES bounded to the biosorbent surface. The surface areas of chrysin, tannic acid and TA-CHY were 5.262, 2.78 and 5.913 m^2/g, respectively.

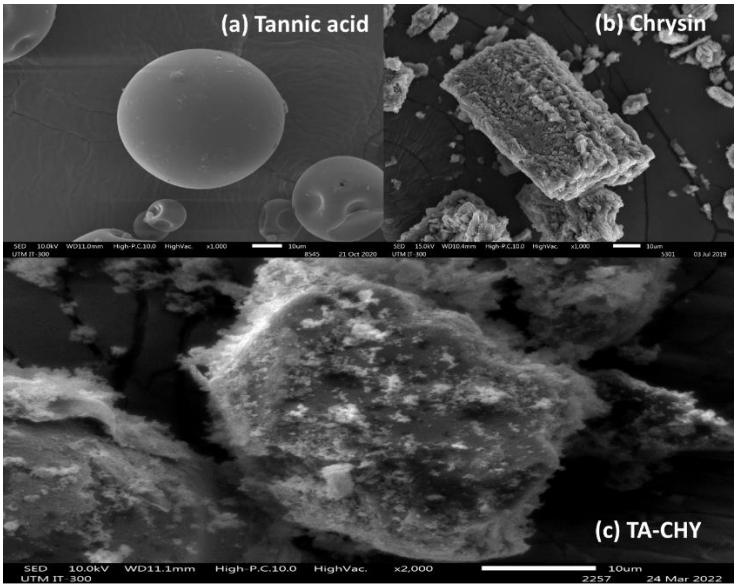

Figure 1. SEM images of (a) tannic acid at ×1000 resolution, (b) chrysin at ×1000 resolution and (c) TA-CHY at ×2000 resolution

Table 1. EDX results of TA-CHY, chrysin and tannic acid

Element	Weight Percentage (%)		
	TA-CHY	Chrysin	Tannic Acid
C	52.9	71.6	55.4
O	34.0	28.4	44.6
Si	6.80	-	-
N	6.30	-	-

Fourier transform infrared spectroscopy (FTIR) study

FTIR spectroscopy is a powerful analytical method that provides useful information on the functional groups that exist in the materials under investigation. The FTIR method was utilized in this investigation to analyze the functional groups of TA-CHY, chrysin, and tannic acid, as shown in Figure 2 (A). The FTIR spectra of chrysin showed multiple peaks at 1447.23-1496.74 and 3006.96 cm^{-1}, which were ascribed to aromatic ring C=C stretching and C-H stretching, respectively. Tannic acid and TA-CHY, on the other hand, displayed multiple C=C stretching bands of aromatic ring with vibration wavelengths of 1444.48-1607.43 and 1447.47-1600.36 cm^{-1}, respectively [19]. Furthermore, the C-O stretching, =C-H bending, C=O stretching, and C-O-C stretching were related with the peaks of chrysin at 1243.55, 839.19, 1649.34, and 1164.63 cm^{-1}, respectively. Many functional groups, including C=O, O-H, O=C-O-C, and C-O, were detected in tannic acid, with peaks emerging at 1703.14 (C=O stretching), 3273.99 (O-H stretching), 1310.56 (aromatic O=C-O-C stretching), and 1181.64 cm^{-1} (C-O stretching of aromatic alcohol). Several notable vibration frequencies were also detected in the FTIR spectra of TA-CHY: Sp^2 C=N double bond stretching measured at 1700.9 cm^{-1}, phenolic O-H bending measured at 1329.74 cm^{-1}, Si-OR stretching measured at 1038.77-1071.21 cm^{-1}, O-H stretching measured at 3152.64 cm^{-1}, and aromatic alcohol C-O stretching measured at 1198 cm^{-1}. This showed that some significant functional groups found in TA-CHY include the benzene ring, the O-H, the C=N, and the C=O groups, may improve the contacts between the sorbent and the sorbate via hydrophobic interaction, cation-π interaction, π-π interaction/aromatic stacking, and hydrogen bonding [20, 21]. Furthermore, the presence of a silanol group in TA-CHY showed that APTES were attached to the biosorbent surface to aid in the deposition of chrysin on tannic acid, as shown in Figure 1 (c). The discussed FTIR data are listed and tabulated in Table 2.

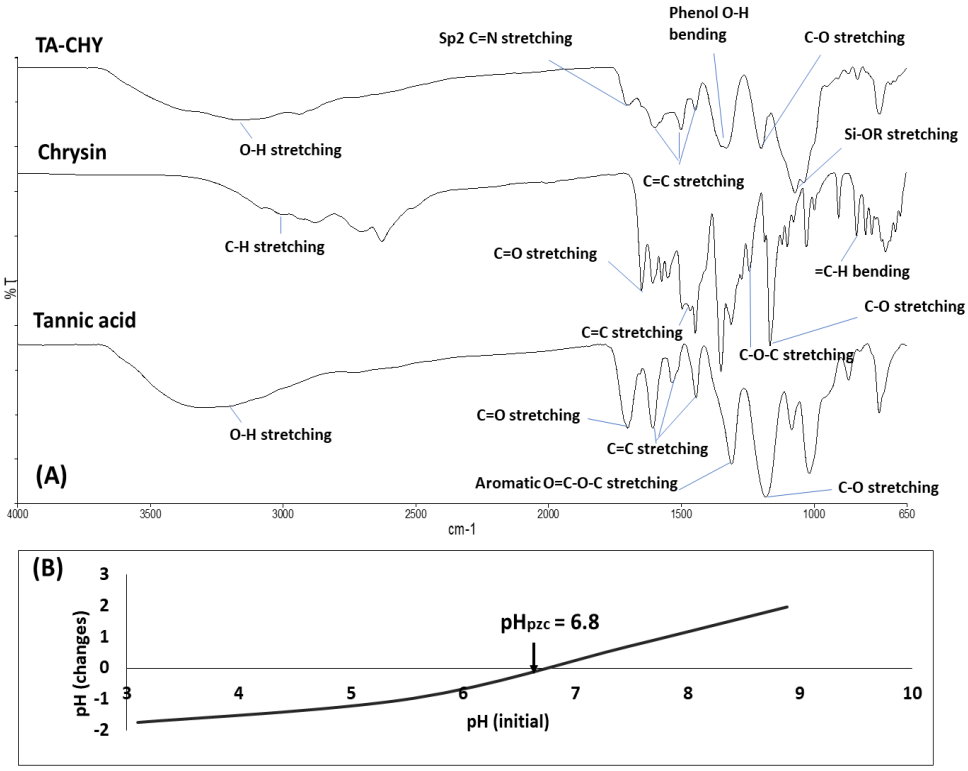

Figure 2. FTIR spectra of TA-CHY, chrysin and tannic acid (A); Graph of point of zero charge (B)

Table 2. FTIR results of chrysin, TA-CHY and tannic acid

Sample	Wavenumber (cm^{-1})	Functional group	Vibration
Chrysin	1243.55	Aromatic alcohol	C-O stretching
	839.19	Alkene	=C-H bending
	1447.23, 1465.50, 1496.74	Aromatic ring	C=C stretching
	3006.96	Aromatic ring	C-H stretching
	1649.34	Ketone	C=O stretching
	1164.63	Ether	C-O-C stretching
TA-CHY	1700.9	Imine	Sp^2 C=N double bond stretching
	1447.47, 1501.12, 1600.36	Aromatic ring	C=C stretching
	1329.74	Aromatic alcohol	Phenol O-H bending
	1038.77, 1071.21	Silanol	Si-OR stretching
	3152.64	Carboxylic acid	O-H stretching
	1198	Aromatic alcohol	C-O stretching
Tannic acid	1310.56	Ester	Aromatic O=C-O-C stretching
	1607.43, 1444.48, 1533.71	Aromatic ring	C=C stretching
	1703.14	Ester	C=O stretching
	1181.64	Aromatic alcohol	C-O stretching
	3273.99	Carboxylic acid	O-H stretching

Point of zero charge (pH_{pzc}) determination

It is vital for scientists to investigate the pH_{pzc} of a biosorbent surface in order to identify its relative surface charges. When the surface charge of a biosorbent surface is thoroughly studied, interactions (mostly electrostatic interactions) between analytes and the biosorbent

surface may be easily manipulated. When the solution pH is greater than pH_{pzc}, the biosorbent surface showed negative charges, and vice versa when the solution pH falls below the pH_{pzc}. When the pH solution was equal to pH_{pzc}, the biosorbent surface was in a neutral condition. Figure 2 (B) depicts a curve formed by graphing $pH_{changes}$ vs $pH_{initial}$. pH_{pzc} of TA-CHY (pH 6.8) which was identified using the intersection point of the curve at axis-x. At solution pH of 6.8, TA-CHY exhibited a neutral charge. Positive charges were present on the biosorbent surface when the solution pH is less than 6.8. The biosorbent surface, on the other hand, exhibited negative charges when the solution pH exceeded 6.8.

Method optimisation

A series of water samples loaded with PCA and VA at a concentration of 0.5 mg/L were prepared and employed for method optimisation. The involved parameters included type of desorption solvent, extraction time, desorption time, solution pH, NaCl addition, biosorbent mass and dispersant solvent. One variable at a time method was used to optimise these parameters.

Effect of type of desorption solvent

In this study, a total of four different organic solvents, including toluene, hexane, ethanol, and methanol, were tested for their potential to desorb PCA and VA. The solvents were then divided into two groups: non-polar solvents (toluene and hexane) and polar solvents (methanol and ethanol). Figure 3(a) demonstrates that methanol has the greatest desorption ability when compared to other solvents. According to Musa et al. [22], methanol was the optimum desorption solvent for PAs with moderate polarity (log P of PCA= 0.82; log P of VA= 1.35 [23]. During this study, the following extraction variables were kept constant: ethanol as the dispersant solvent, solution pH of 8, extraction duration of 1 min, desorption time of 15 min, biosorbent mass of 0.1 g, and 0% NaCl addition (w/v).

Effect of extraction time

Vortex agitation was used for this extraction procedure with the goal to enhance the mass-transfer rate of analytes to the biosorbent surface and shorten the equilibrium period. The extraction time was varied between 0.5 and 4 min with the desorption time set at 15 min whereby methanol was used as the desorption solvent, 0.1 g of biosorbent mass, solution pH 8, 0% NaCl addition (w/v), and ethanol as the dispersant solvent. As seen in Fig. 3 (b), PCA recovery increased from 0.5 to 1 min. The PCA recovery responses decreased and stayed virtually consistent in the 2 to 4 min range. In contrast, VA recovery improved dramatically from 0.5 to 1 min. After that, VA recovery responses fell and stayed steady between 2 and 3 min, followed by another decline at 4 min. The extracted analytes then reached their maximum levels at 1 min. After 1 min, the biosorbent may suffer from active site saturation, resulting in a decline of analyte recovery responses. As a result, 1 min was chosen as the optimal extraction time.

Effect of desorption time

Ultrasonication was utilized to help in the desorption of sorbates from a sorbent by enhancing mechanical agitation and improving mass transfer at the solid-liquid interface. Between 5 and 20 min, the effect of desorption time on sorption recovery was examined (Fig. 3(c)). Other experimental settings were held constant during this study: extraction time was 1 min, desorption solvent was methanol, solution pH was 8, dispersant solvent was ethanol, and the amount of NaCl added was 0% (w/v). Under these conditions, PCA's responses were steady for the first 10 min, then increased at 15 min, followed by a slight decrease at 20 min. The recovery responses for VA climbed gradually from 5 to 15 min, then dropped at 20 min. Both VA and PCA responses decreased after 15 min of desorption processes, suggesting that some of the analytes were re-sorbed by the TA-CHY. Therefore, 15 min was chosen as the desorption time for this research.

Effect of solution pH

As solution pH can have a substantial influence on the existing form of analytes and surface charges of a biosorbent surface which is crucial in an extraction process. The effect of solution pH on PCA and VA recovery was examined in this study throughout a pH range of 1 to 8 (Fig. 3(d)). Other experimental parameters had 15 min of desorption time, methanol as the desorption solvent, 0.1 g of biosorbent mass, 1 min of extraction time, 0% NaCl addition (w/v), and ethanol as the dispersant solvent. Particularly, when the pH increased from 1 to 2, the VA recovery responses increased significantly. The recovery response of VA reduced dramatically and progressively after pH 3 solution. The recovery responses of PCA, on the other hand, were practically consistent from pH 1 to 4. It showed an increasing trend at pH 6 solution before dropping at pH 8 solution. As a result, VA and PCA responded optimally at solution pH levels of 2 and 6, respectively. When compared to PCA (lower hydrophobicity with log P of 0.82 [23], VA (greater hydrophobicity with log P of 1.35 [23] indicated a substantial preference for the positively charged TA-

CHY biosorbent at solution pH 2. This might be attributed to greater cation-π, hydrophobic interactions and π-π interactions between them [24]. In research performed by Musa et al., a higher hydrophobicity analyte, *p*-hydroxybenzoic acid (log P of 2.27) was also found more favorable to a hydrophobic graphene-based adsorbent than a lower hydrophobicity analyte, PCA [22]. In addition, the presence of an extra methyl group in VA might increase electron density in the aromatic ring, resulting in a greater electrostatic affinity with the positively charged biosorbent [25]. This stronger attraction led to a more stable and beneficial cation-π interaction. Other possible interaction mechanisms were primarily driven by hydrogen bonding between the biosorbent's silanol/carbonyl/hydroxyl/amino groups and the analytes' carbonyl/hydroxyl groups. Furthermore, both PCA and VA have pK_a values around 4.2. This might explain why the best solution pH for PCA recovery was pH 6, since there was an electrostatic contact between the deprotonate PCA and the positively charged TA-CHY biosorbent.

Effect of NaCl addition

Salt addition may have a major influence on the sorption process. As salt increases a solution's ionic strength, it may impact the sorbate sorption efficiency and selectivity. For example, when the ionic strength of a solution changed, the electrostatic connections between the analytes and biosorbent were disrupted and weakened. Figure 3(e) shows that when no NaCl salt (0%, v/w) was added to the sample solution, both PCA and VA showed the best recovery responses. Their recovery responses decreased considerably when 1% and 2% (w/v) of NaCl were added to the sample solutions. This is because the additional NaCl salt may compete with the analytes for binding sites on the TA-CHY surface. Therefore, 0% (v/w) NaCl salt addition was set for this method. Other experimental settings used in this optimisation were 1 min of extraction time, 15 min of desorption time, methanol as the desorption solvent, solution pH 2, ethanol as the dispersant solvent and 0.1 g of biosorbent mass.

Effect of mass of biosorbent

The effect of TA-CHY mass on PCA and VA recovery was investigated by increasing the mass from 0.10 g to 0.25 g while keeping the other experimental parameters constant: extraction time = 1 min, desorption solvent = methanol, desorption time = 15 min, solution pH = 2, dispersant solvent = ethanol and NaCl addition = 0% (w/v). The recovery responses of VA indicated an increasing trend from 0.10 to 0.20 g, followed by a drop at 0.25 g in Fig. 3(f). PCA showed a rising tendency from 0.10 to 0.15 g, followed by a drop after 0.20 g. The availability of more analyte sorption sites resulted in an increase in recovery responses with increasing amounts of biosorbent. Therefore, the best biosorbent mass for VA and PCA were 0.20 g and 0.15 g, respectively. The biosorbent mass for the subsequent study was determined to be 0.15 g. The observed reduction in the recovery response of PCA and VA at larger biosorbent mass ranges may be due to the saturation of the TA-CHY adsorption sites and the desorption of analytes back to the bulk phase.

Effect of type of dispersant solvent

In this study, a dispersant solvent was used to promote biosorbent dispersion in a sample solution. Extraction time, desorption solvent, desorption time, solution pH, NaCl addition, and mass of biosorbent were all designated as 1 min, methanol, 15 min, pH 2, 0% (w/v), and 0.15 g biosorbent, respectively. Acetonitrile, ethanol, and methanol were chosen to investigate their potential as dispersant solvents in the newly designed d-μ-SPE (Figure 3(g)). Among these solvents, the use of ethanol as a dispersant solvent provided the highest analyte recovery results. Ethanol, isopropanol, and acetonitrile had log P values of -0.34, 0.05, and -0.54, respectively. The hydrophilicity of these solvents was organized based on their Log P values: isopropanol, ethanol and acetonitrile in ascending order. Since isopropanol is less hydrophilic, it may cause inadequate dispersion of TA-CHY in the sample, thus resulting in unequal interaction between the biosorbent and the analytes. While acetonitrile is more hydrophilic, it may interact negatively with the hydrophobic component of TA-CHY, resulting in poorer biosorbent-analyte interactions. However, because of the modest hydrophilicity, ethanol was able to adequately disseminate the CHT-TA in the sample. The enhanced dispersion was due to its high compatibility with TA-CHY, which enhanced the interaction of TA-CHY with analytes. Therefore, ethanol was chosen as the dispersant solvent.

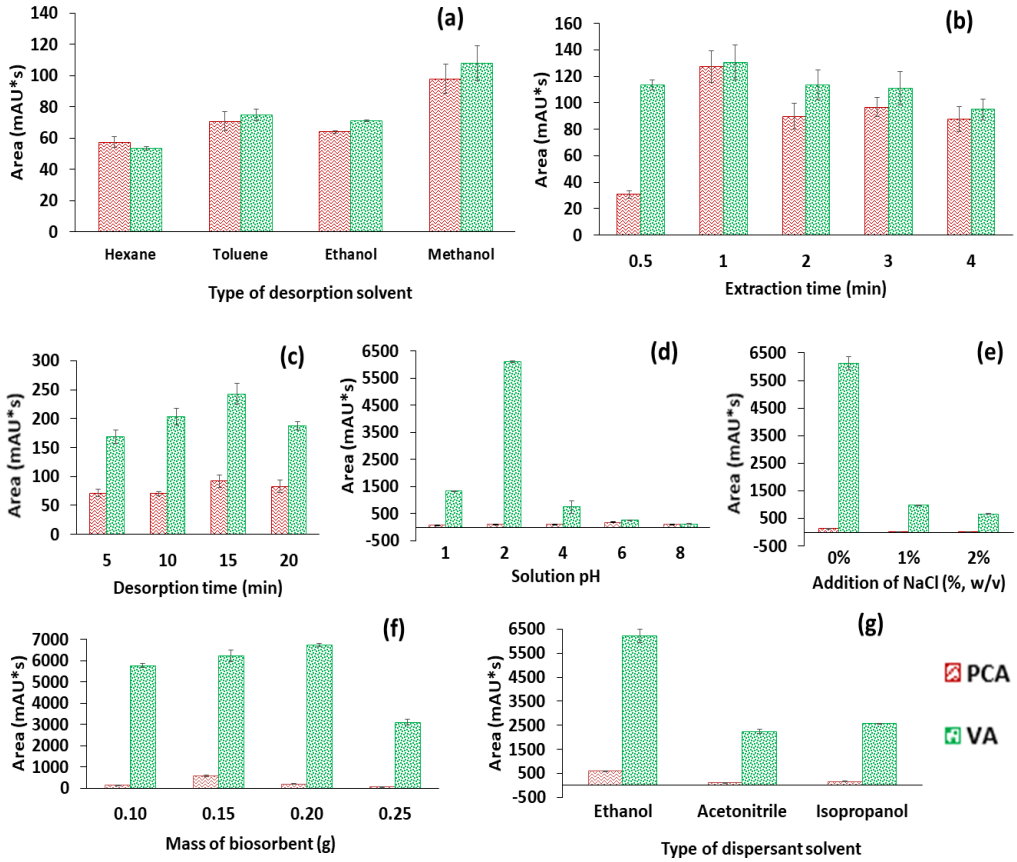

Figure 3. Effects of (a) type of desorption solvent, (b) extraction time, (c) desorption time, (d) solution pH, (e) addition of NaCl, (f) mass of biosorbent and (g) type of dispersant solvent on TA-CHY-d-μ-SPE of VA and PCA

Method validation

Matrix-matched calibration was used to match the matrices of SBH samples and calibration standards to equalize the analyte responses. A series of genuine samples were prepared by spiking PCA and VA in concentrations ranging from 0.5 to 50 mg/L. Both analytes displayed good linearity, with R^2=0.9929 for PCA and 0.9962 for VA (Table 3). LOD values of PCA and VA were 1.37 and 0.81 mg/kg, respectively; while LOQ values of PCA and VA were 4.56 and 2.69 mg/kg, respectively. The approach achieved good RRs and RSDs in the range of 79.38-113.38% and 0.29-9.83%, respectively. To summarize, the approach was shown to be adequate for the determination of PCA and VA in SBH samples with good analytical features.

Table 3. Analytical parameters of the developed method

Analytical Parameter	PCA	VA
Linearity range (mg/L)	0.5-50	0.5-50
LOD (mg/kg), S/N= 3	1.37	0.81
LOQ (mg/kg), S/N= 10	4.56	2.69
Intraday study (*n*=3)		
Spiked concentration at 5 mg/L		
RR (%); RSD (%)	79.38; 2.53	88.88; 0.29
Spiked concentration at 40 mg/L		

Analytical Parameter	PCA	VA
RR (%); RSD (%)	113.12; 9.83	113.38; 7.07
Intraday study (*n*=9)		
Spiked concentration at 5 mg/L		
RR (%); RSD (%)	80.58; 3.01	87.86; 3.43
Spiked concentration at 40 mg/L		
RR (%); RSD (%)	90.49; 9.92	99.45; 10.83

Real sample analysis

The d-μ-SPE approach, which used TA-CHY as a biosorbent, was expanded to analyze PCA and VA in genuine SBH samples collected from Malaysia's Malacca, Perlis, Perak, and Selangor states. Table 4 summarizes the measure quantities of both analytes reported for these SBH samples and their comparison with other honey products. According to the data presented in Table 4, there appears to be a trend showing that SBH samples had greater levels of PCA and VA [26, 27]. The greatest amount of PCA were found in H9 (Greece), SBH sample 2 (Perak, Malaysia), and SBH sample 3 (Perlis, Malaysia), with amount of 16.777, 7.69, and 10.67 mg/kg, respectively. SBH samples 2 (Perak, Malaysia), B and D (Santa Catarina State, Brazil) had the greatest levels of VA, at 11.97, 4.21, and 3.46 mg/kg, respectively. SBHs showed to benefit honey consumers because VA has anti-obesity, anti-diabetic and cardio-protective properties, whilst PCA has anti-inflammatory, antioxidant, anti-microbial, anti-apoptotic, anti-hyperglycemic and anti-proliferative properties [28].

Table 4. Results of PCA and VA analysis in real SBH sample and their comparison with other honey products

Sample, Source	Sample Code	Found Amount (mg/kg ± SD)		Reference
		PCA	VA	
SBH, Malacca, Malaysia	1	2.31±0.26	ND	This study
SBH, Perak, Malaysia	2	7.69±0.86	11.97±1.33	
SBH, Perlis, Malaysia	3	10.67±1.20	ND	
SBH, Selangor, Malaysia	4	3.88±0.43	ND	
SBH, Santa Catarina State, Brazil	A	0.38±0.5	ND	[29]
	B	0.07±0.1	4.21±1.0	
	C	ND	ND	
	D	ND	3.46±0.7	
	E	0.53±1.0	2.78±1.4	
	F	ND	3.36±1.4	
	G	ND	0.96±0.1	
	H	0.4±0.1	ND	
Honey and SBH, different states of Brazil	*Apis mellifera* honey (control)	$0.016\pm0.002\times10^{-3}$	NM	[30]
	SBH1	ND	NM	
	SBH2	ND	NM	
Honey, different regions of Greece	H1	0.649±0.015	0.225±0.006	[31]
	H5	0.346±0.025	0.236±0.002	
	H11	0.300±0.022	0.245±0.011	
	H8	0.590±0.021	0.103±0.001	
	H9	16.777±0.780	0.307±0.014	
	H7	3.258±0.024	0.149±0.002	
	H4	4.140±0.131	0.262±0.008	
	H10	5.967±0.057	0.376±0.002	
	H6	3.058±0.111	0.340±0.013	
	H3	2.394±0.050	0.237±0.007	
	H2	1.046±0.027	0.201±0.005	

Sample, Source	Sample Code	Found Amount (mg/kg ± SD)		Reference
		PCA	VA	
	H12	0.303±0.007	0.092±0.002	
Honey, Xinjiang, China	1	NM	0.042±0.001	[32]
	2	NM	0.021±0.001	
	3	NM	0.031±0.001	
	4	NM	0.052±0.001	
	5	NM	0.031±0.001	
	6	NM	ND	
	7	NM	ND	
	8	NM	0.022±0.001	
	9	NM	0.038±0.001	
	10	NM	ND	

ND= not detectable; NM= not mentioned

Method comparison

Table 5 compares several sample preparation techniques, including this approach, solvent extraction, G-Fe_3O_4-MSPE, and HLB SPE. These techniques were reviewed and compared in terms of LOD, LOQ, linearity range, RSD, and RR. Remarkably, HLB SPE exhibited an outstanding sensitivity with the lowest LOD (0.0002 µg/kg) and LOQ values (0.0006 µg/kg), allowing the reliable detection and quantification of trace VA concentrations. Secondly, G-Fe_3O_4-MSPE allowed for facile sorbent collection by using an external magnetic field while retaining its good analytical properties. Moreover, G-Fe_3O_4-MSPE also used the least amount of toxic organic solvent (~500 µL) during the sample preparation stage, as compared to solvent extraction (>100 mL), HLB SPE (>15mL) and this method, TA-CHY-d-µ-SPE (<4 mL). However, TA-CHY-d-µ-SPE demonstrated comparable sensitivity and accuracy to solvent extraction but used less hazardous organic solvent than solvent extraction. All techniques mentioned in Table 5 demonstrated adequate accuracy, with RSD values of less than 10.83%, thus emphasizing their precision and consistency between replicates. Overall, each technique has its own set of benefits and drawbacks. TA-CHY-d-µ-SPE is considered sufficient and effective in accomplishing its intended purpose in the analysis of PAs in SHB.

Table 5. Comparison with other published sample preparation methods

Method	Sample	Analyte	LOD (µg/kg)	LOQ (µg/kg)	Linearity range	RSD (%)	RR (%)	Reference
TA-CHY-d-µ-SPE	SBH	PCA	1.37	4.56	0.5-50 mg/L	2.53-9.92	79.38-113.12	This study
		VA	0.81	2.69		0.29-10.83	87.86-113.38	
HLB SPE	Honey	PCA	NM	NM	NM	NM	NM	[32]
		VA	0.0002	0.0006	0.001-0.50 mg/L	2.4-5.1	78.5-82.9	
Solvent extraction	Honey	PCA	0.99	3.00	NM	NM	>85	[33]
		VA	0.89	2.71	NM	NM		
G-Fe_3O_4-MSPE	SBH	PCA	0.14	0.46	3-50 mg/kg	0.3-8.5	78.8-107.5	[22]
		VA	NM	NM	NM	NM	NM	

*NM= not mentioned; SPE= solid phase extraction; HLB= Hydrophilic-Lipophilic Balance; G-Fe_3O_4= graphene-magnetite composite; MSPE= magnetic solid phase extraction

Analytical Eco-scale

An analytical Eco-scale calculation [18] was performed to evaluate the eco-friendliness of the developed d-μ-SPE method combined with the use of TA-CHY biosorbent. This covered several stages of a single sample analysis, namely biosorbent preparation, sample preparation and instrumental detection. The calculation was based on the total score obtained from a deduction of total penalty points from 100. The penalty points were given for using less green reagent, instrument, high waste volume and occupational hazard. 100 score of analytical Eco-scale indicated a method as ideal green analysis, whereas score above 75 was excellent, score between 74 and 50 suggested the method as an acceptable green analysis, and score at below 50 indicated inadequate method. This method received a total score of 71, suggesting that it is an acceptable green analytical approach. The calculation details were shown in Table 6.

Table 6. Analytical Eco-scale calculation

Reagent	**Penalty Point**			
	Amount (Point)	**Number of Pictogram (Point)**	**Signal Word (Point)**	**Calculated Point**
Chrysin	<10 g(1)	0(0)	None(0)	1×0×0=0
3-aminopropyltriethoxysilane	<10 mL(1)	2(2) 	Danger(2)	1×2×2=4
Tannic acid	10 g(1)	0(0)	None(0)	1×0×0=0
Sodium hydroxide	<10 g(1)	1(1) 	Danger(2)	1×1×2=2
Ethanol	10-100 mL (2)	2(2) 	Danger(2)	2×2×2=8
Phosphoric acid	<10 mL(1)	2(2) 	Danger(2)	1×2×2=4
Methanol	<10 mL(1)	3(3) 	Danger(2)	1×3×2=6
Potassium dihydrogen phosphate	<10 g(1)	0(0)	None(0)	1×0×0=0
Water	10-100 mL(2)	0(0)	None(0)	2×0×0=0
Total point				**24**
Instrument		**Energy used per sample (kWh) (point)**		**Calculated point**
HPLC-UV		≤1.5(1)		1
Centrifuge		≤1.5(1)		1
Vortex mixer		≤0.1(0)		0
Vacuum pump		≤0.1(0)		0
Sonicator		≤0.1(0)		0
Total point				**2**
Other				
Waste		<10 mL(3)		3
Occupational hazard		Hermetization in analytical process(0)		0
Total point				**3**
Total penalty point				24+2+3=29
Analytical Eco-Scale total score				100-29=71 **(acceptable)**

Conclusion

In conclusion, our investigation on the newly developed biosorbent, TA-CHY for the extraction of PAs offered substantial insights. CHY-TA had been successfully prepared, characterised and applied for d-μ-SPE of VA and PCA, with higher selectivity towards VA via strong cation-π, hydrophobic interactions and π-π interactions with CHY-TA. The method was extended for the analysis of PCA and VA in Malaysian SBHs. It demonstrated good analytical performance, with LODs ranging from 0.81 to 1.37 mg/kg, LOQs ranging from 2.69 to 4.56 mg/kg, RRs ranging from 79.38% to 113.38%, and RSDs ranging from 0.29% to 10.83%. It also proved its capability to stand alone as a new green analytical method (analytical Eco-scale = 71; acceptable green analysis). This research also contributed to the advancement of analytical methods for studying phenolic acids in stingless bee honey, emphasizing the potential for broader applications in honey analysis and quality assessment.

Acknowledgement

The authors would like to acknowledge the Ministry of Higher Education Malaysia for Fundamental Research Grant Scheme (FRGS) funding with reference code FRGS/1/2019/STG01/UTM/01/2 (Reference No: PY/2019/02237; vote number R.J130000.7854.5F268) and Universiti Teknologi Malaysia (UTM) for the UTM Fundamental Research (UTM FR) funding (Reference No: PY/2019/01834; vote number Q. J130000.2554.21H56). The authors declared no conflict of interest.

References

1. Raja Nurfatin, R. M. Y., Norhayati, M. K., Mohd Fairulnizal, M. N., Hadi, N., Abdul Manam, M., Mohd. Zin, Z. and Yusof, H. M. (2021). The physicochemical, sensory evaluation and glycemic load of stingless bee honey and honeybee honey. *Food Research,* 5(1): 99-107.
2. Najeeb, I. M. A., Norhayati M. K., Zaharah, H., Mohd Isa, N. S., Nur Nadrah, M. R. and Yusof H. M. (2022). Physicochemical properties, sensory acceptance and glycaemic index of processed stingless bee honey and processed honeybee honey. *Food Research*, 6(6): 103-110.
3. Luo, D., Mu, T. H. and Sun, H. N. (2021). Profiling of phenolic acids and flavonoids in sweet potato (*Ipomoea batatas L.*) leaves and evaluation of their anti-oxidant and hypoglycemic activities. *Food Bioscience*, 39: 100801.
4. Saleem, A., Akhtar M. F., Sharif, A., Akhtar, B., Siddique, R., Ashraf, G. M., Alghamdi, B. S. and Alharthy, S. A. (2022). Anticancer, cardio-protective and anti-inflammatory potential of natural-sources-derived phenolic acids. *Molecules*, 27(21): 7286.
5. Valverde, S., Ares, A. M., Stephen Elmore, J. and Bernal, J. (2022). Recent trends in the analysis of honey constituents. *Food Chemistry*, 387: 132920.
6. Gałuszka, A., Migaszewski, Z. and Namieśnik, J. (2013). The 12 principles of green analytical chemistry and the SIGNIFICANCE mnemonic of green analytical practices. *TrAC Trends in Analytical Chemistry*, 50: 78-84.
7. Pascual-Maté, A., Osés, S. M., Fernández-Muiño, M. A. and Sancho, M. T. (2018). Analysis of polyphenols in honey: extraction, separation and quantification procedures. *Separation & Purification Reviews*, 47(2): 142-158.
8. Anastassiades, M., Lehotay, S. J., Štajnbaher, D. and Schenck, F. J. (2003). Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. *Journal of AOAC international*, 86(2): 412-431.
9. Ghorbani, M., Aghamohammadhassan, M., Ghorbani, H. and Zabihi, A. (2020). Trends in sorbent development for dispersive micro-solid phase extraction. *Microchemical Journal*, 158: 105250.
10. Ng, N. T., Abdul Keyon, A. S., Wan Ibrahim, W. A., Sanagi, M. M., Sutirman, A. A. and Mohd Marsin, F. (2023). Amino-functionalised chrysin as adsorbent in dispersive micro-solid phase extraction of selected heavy metal ions from stingless bee honey. *Journal of Food Composition and Analysis*, 123: 105561.
11. Ścigalski, P. and Kosobucki P. (2020). Recent materials developed for dispersive solid phase extraction. *Molecules*, 25(21): 4869.

12. Hang, N., Yang, Y., Zhang, Y. Y., Zhao, W. N., Tao, J. and Li, S. Q. (2023). Magnetic cork composites as biosorbents in dispersive solid-phase extraction of pesticides in water samples. *Analytical Methods*, 15(29): 3510-3521.
13. Kachangoon, R., Vichapong, J., Santaladchaiyakit, Y. and Srijaranai S. (2022). Green fabrication of *Moringa oleifera* seed as efficient biosorbent for selective enrichment of triazole fungicides in environmental water, honey and fruit juice samples. *Microchemical Journal*, 175: 107194.
14. Adlnasab, L., Ezoddin, M., Shabanian, M. and Mahjoob, B. (2019). Development of ferrofluid mediated CLDH@Fe_3O_4@Tanic acid- based supramolecular solvent: Application in air-assisted dispersive micro solid phase extraction for preconcentration of diazinon and metalaxyl from various fruit juice samples. *Microchemical Journal*, 146: 1-11.
15. Abd Ali, L. I., Wan Ibrahim, W. A., Sulaiman, Azli, Kamboh, M. A. and Sanagi, M. M. (2016). New chrysin-functionalized silica-core shell magnetic nanoparticles for the magnetic solid phase extraction of copper ions from water samples. *Talanta*, 148: 191-199.
16. Shen, N., Wang, T. F., Gan, Q., Liu, S., Wang, L. and Jin, B. (2022). Plant flavonoids: Classification, distribution, biosynthesis, and antioxidant activity. *Food chemistry*, 383: 132531.
17. Pyrzynska, K. and Pękal, A. (2011). Flavonoids as analytical reagents. *Critical Reviews in Analytical Chemistry*, 41(4): 335-345.
18. Gałuszka, A., Migaszewski, Z. M, Konieczka, P. and Namieśnik, J. (2012). Analytical Eco-Scale for assessing the greenness of analytical procedures. *TrAC Trends in Analytical Chemistry*, 37: 61-72.
19. Liu, L. L., Ge, C., Zhang, Y., Ma, W. R., Su, X., Chen, L., Li, S. B., Wang, L., Mu, X. J. and Xu, Y. (2020). Tannic acid-modified silver nanoparticles for enhancing anti-biofilm activities and modulating biofilm formation. *Biomaterials Science*, 8(17): 4852-4860.
20. Abebe, M.W. and Kim, H. (2022). Methylcellulose/tannic acid complex particles coated on alginate hydrogel scaffold via pickering for removal of methylene blue from aqueous and quinoline from non-aqueous media. *Chemosphere*, 286: 131597.
21. Park, S. H., Choi, S. J., Lee, Y. K., Jho, Y. S., Kang, S. T. and Hwang, D. S. (2023). Cation− π interactions contribute to hydrophobic humic acid removal for the control of hydraulically irreversible membrane fouling. *Environmental Science & Technology*, 57(9): 3853-3863.
22. Musa, M., Wan Ibrahim, W. A., Mohd Marsin, F., Abdul Keyon, A. S. and Rashidi Nodeh, H. (2018). Graphene-magnetite as adsorbent for magnetic solid phase extraction of 4-hydroxybenzoic acid and 3,4-dihydroxybenzoic acid in stingless bee honey. *Food Chemistry*, 265: 165-172.
23. Galanakis, C. M., Goulas, V., Tsakona, S. Manganaris, G. A. and Gekas, V. (2013). A knowledge base for the recovery of natural phenols with different solvents. *International Journal of Food Properties*, 16(2): 382-396.
24. Yamada, S. (2020). Cation–π interactions in organic crystals. *Coordination Chemistry Reviews*, 415: 213301.
25. Kim, S. H., Gupta, N. K., Bae, J. Y. and Kim, K. S. (2020). Structural variations and generation of binding sites in Fe-loaded ZSM-5 and silica under the effect of uv-irradiation and their role in enhanced BTEX abatement from gas streams. *Journal of Hazardous Materials*, 384: 121274.
26. Shamsudin, S., Selamat, J., Abdul Shomad, M., Ab Aziz, M. F. and Haque Akanda, M. J. (2022). Antioxidant properties and characterization of *Heterotrigona itama* honey from various botanical origins according to their polyphenol compounds. *Journal of Food Quality*, 2022: 2893401.
27. Zulkifli, N.A., Hassan, Z., Mustafa, M. Z., Wan Azman, W. N., Hadie Hanim, S. N., Ghani, N. and Mat Zin, A. A. (2023) The potential neuroprotective effects of stingless bee honey. *Frontiers in Aging Neuroscience*, 14: 1048028.
28. Rashmi, H. B. and Negi, P. S. (2020). Phenolic acids from vegetables: A review on processing stability and health benefits. *Food Research International,* 136: 109298.
29. Biluca, F. C., da Silva, B., Caon, T., Mohr, E. T., Bramorski, V., Guilherme, N., Gonzaga, L., Valdemiro, V., Luciano, M., Gustavo, F., Roseane,

D. and Eduardo, M. (2020). Investigation of phenolic compounds, antioxidant and anti-inflammatory activities in stingless bee honey *(Meliponinae). Food Research International*, 129: 108756.

30. Moreira, F. I. N., de Medeiros, L. L., de Carvalho, L. M., Olegario, L. S., de Sousa Galvão, M., da Franca, S. A. M., Bezerra, T. K. A., dos Santos Lima, M. and Madruga, M. S. (2023). Quality of brazilian stingless bee honeys: *Cephalotrigona Capitata/Mombucão* and *Melipona Scutellaris Latrelle/Uruçu. Food Chemistry*, 404: 134306.
31. Spilioti, E., Jaakkola, M., Tolonen, T., Lipponen, M., Virtanen, V., Chinou, L., Kassi, E., Karabournioti, S. and Moutsatsou, P. (20140. Phenolic acid composition, antiatherogenic and anticancer potential of honeys derived from various regions in Greece. *PloS One*, 9(4): e94860.
32. Wang, Y., Xing, L. J., Zhang, J. L., Ma, X. N. and Weng, R. (2023). Determination of endogenous phenolic compounds in honey by HPLC-MS/MS. *LWT*, 114951.
33. Puścion-Jakubik, A., Karpińska, E., Moskwa, J. and Socha, K. (2022). Content of phenolic acids as a marker of polish honey varieties and relationship with selected honey-quality-influencing variables. *Antioxidants*, 11(7): 1312.