ASSESSMENT OF PESTICIDE RESIDUES AND HEAVY METALS AS WELL AS INVESTIGATION OF HOUSEHOLD METHODS FOR PESTICIDE RESIDUE ELIMINATION FROM IMPORTED DATE FRUITS IN MALAYSIAN MARKET

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Thesis submitted in fulfilment for the degree of
DOCTOR OF PHILOSOPHY IN
SCIENCE AND TECHNOLOGY

UNIVERSITI SAINS ISLAM MALAYSIA

April 2022

AUTHOR DECLARATION

I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledged.

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ACKNOWLEDGEMENTS

In the name of ALLAH (s.w.t), all praise is due to Him, the only creator, WHO bestowed us the knowledge, strength, and perseverance to bring this work to fruition, and may His blessing be upon His prophet Mohammad (s.a.w), peace be upon him.

I respectfully express my deepest sense of gratitude to my supervisor Associate Prof Dr. Che Wan Zanariah for her guidance and generous help throughout the course of this work and in the preparation of this thesis.

I am pleased to express my deepest tribute to my co- supervisor Associate Prof Dr. Mohammed Sukri Hassan for his help throughout the course of this work and in the preparation of this thesis.

My gratitude also goes to Dean of the Faculty of Science and Technology for allowing me to use departmental facilities throughout my research.

I would like to thank all technicians and laboratory assistants for assistance, especially, En. Syamsul Kamar Muhamad, Pn. Normah Haron and En. Muhammad Izzat Redzuwan.

Finally, I am also grateful to Libyan embassy (Malaysia) for providing me a fellowship during my candidature.

ABSTRAK

Bahan toksik yang mencemari alam sekitar boleh memudaratkan kesihatan manusia. Pestisid dan logam berat adalah toksin utama yang menimbulkan ancaman kepada alam semula jadi. Pengembangan dan pengesahan kaedah-kaedah untuk mengekstrak dan menganalisis sisa bahan pencemar alam sekitar seperti pestisid dan logam berat dalam sayur-sayuran dan buah-buahan telah mendapat perhatian untuk memastikan bahawa kadar bahan pencemar di dalamnya berada dalam had yang dibenarkan untuk melindungi kesihatan pengguna. QuEChERS dengan penimbal asetat (AOAC) telah berjaya diubahsuai dan dioptimumkan untuk pengekstrakan matriks buah kurma dan analisis sisa pestisid dengan menaplikasikan Perisian Minitab 17.0 dan program reka bentuk penapisan Plakett-Burman (P-B). Kaedah yang telah diubahsuai menunjukkan pemulihan tinggi dan kesan matriks rendah (di bawah 20) untuk tiga pestisid yang diuji, dua organofosforus (dimethoate dan chlopyrifos) dan satu karbamat (carbendazim). Prestasi kaedah yang diubahsuai berkenaan lebih baik daripada kaedah tanpa penimbal asal dari segi pemulihan pestisid. HPLC-DAD telah dibangun dan dioptimumkan untuk pengesanan serentak pestisid di dalam sampel buah kurma yang diekstrak dengan kaedah pengekstrakan dan pembersihan dengan penimbal asetat yang diubahsuai (QuEChERS). Kaedah ini disahkan dengan menentukan parameter-parameter analisis dan statistik berikut: kelinearan, had pengesanan (LOD), had kuantifikasi (LOQ), dan ketepatan kaedah. Pemulihan (%) didapati berada di antara 101-104.2 %. Di antara 20 sampel yang dikaji, dimethoate telah dikesan dalam 13 sampel (65%) dalam julat di antara 0.01059-0.053 mg/kg berat segar. Carbendazim dikesan dalam semua sampel yang dikaji (100 %) dalam julat di antara 0.01485-0.3207 mg/kg berat segar. Chlorpyrifos dikesan dalam 12 sampel (60 %) dalam julat di antara 0.0324-1.543 mg/kg berat segar. Penilaian risiko kesihatan terhadap pestisid-pestisid yang dikaji dikira berdasarkan min kepekatan dalam setiap sampel buah kurma. Walaupun sisa pestisid dalam kebanyakan sampel melebihi MRL Suruhanjaya Eropah, tidak terdapat risiko kesihatan yang berkaitan dengan pestisid-pestisid yang dikaji memandangkan tiada sampel yang melebihi had indeks bahaya kesihatan (HHI) yang boleh diterima (<1) bagi orang dewasa atau kanak-kanak. Keberkesanan beberapa kaedah membasuh untuk menghilangkan sisa pestisid dari buah kurma telah dikaji dan dioptimumkan. Kaedah-kaedah ini merangkumi membasuh dengan air paip, larutan sabun pencuci pinggan dan tiga tahap kepekatan sama ada dengan NaCl, asid asetik, H_2O_2 atau $KMnO_4$ (1 %, 2 %, dan 3 % v/v) dalam dua suhu berbeza, 25°C dan 37°C. Kepekatan pestisid berkurang secara ketara dengan bertambahnya masa sentuhan, suhu dan kepekatan pada buah yang dirawat dengan larutan-larutan pencuci yang berbeza. Kesan basuhan terhadap nilai pemakanan kurma (jumlah gula, jumlah fenolik dan keupayaan antioksidan (aktiviti perencatan DPPH) telah dikaji dan dioptimumkan. Kesemua rawatan menghasilkan kesan negatif terhadap nilai pemakanan. Penurunan nilai pemakanan berlaku secara beransur-ansur dengan bertambahnya masa sentuhan, suhu dan kepekatan larutan pencuci. Satu kaedah penghadaman dan penentuan lapan logam berat dalam isi kurma dan dalam habuk yang melekat pada kurma telah dibangun dan dioptimumkan menggunakan spektrofotometer GFAAS. Purata kepekatan logam berat dalam isi kurma adalah sama dengan nilai global dan mengikut tertib menurun: Fe>Al>Cu>As>Ni>Pb>Sn>Cd. Penilaian risiko yang dikira daripada purata kepekatan logam berat dalam sampel menunjukkan bahawa darjah bahaya (HQ) kurang daripada 1 untuk setiap logam berat yang dikaji mencadangkan bahawa memakan kurma berkenaan tidak menyebabkan risiko kesihatan. Kaedah membasuh buah kurma selama lebih kurang 90 saat pada suhu bilik dapat mengurangkan kadar logam berat dalam kesemua sampel. Membasuh buah kurma juga dapat menghilangkan logam berat dalam julat dari 0.95 % hingga 34.78 %.

الملخص

تاتي المبيدات والمعادن الثقيلة على رأس قائمة السموم البيئية التي تشكل تهديدًا للطبيعة و تعرض صحة الإنسان للخطر لذا أصبح تطوير طرق استخلاص وتحليل الملوثات البيئية المتبقية مثل المبيدات والمعادن الثقيلة في الخضار والفواكه يلقى اهتماما كبيرا للتأكد من أن مستوى الملوثات فيها ضمن الحدود المسموح بها لحماية صحة المستهلكين ,حيث تم تعديل طريقة *QuEChERS (AOAC)* وتحسينها لاستخلاص وتحليل بقايا المبيدات في التمور باستخدام برنامج *Minitab 17.0* و اختبار تصميم الفحص *Plackett-Burman (P-B)*. اظهرت الطريقة المعدلة استرجاعا عاليًا لبقايا المبيدات وتأثيرًا منخفضًا للوسط على دقة القياسات (أقل من ٢٠) للمبيدات الثلاثة التي تم اختبارها ، وهي الديميثويت وكلوربيريفوس *(Ops)* وكاربيندازيم*(Carbamates)*. تفوقت الطريقة المعدلة على الطريقة الأصلية من حيث نسبة استرجاع المبيدات , تم تطوير وتقييس طريقة كروماتوغرافيا السائلة (*HPLC-DAD*) للفصل والتقديرالمتزامن لبقايا المبيدات في عينات فاكهة التمر المستخلصة بطريقة*Acetate Buffered QuEChERS* , تم التحقق من صحة وملائمة الطريقة لحدود التقدر الكيفي والكمي وخطية الطريقة ونسبة الاسترجاع وكانت النسبة المؤية للاسترجاع بين ٩٨-١٠٤٫٢ ٪, تم رصد وتقدير مبيد الديمثويت في ١٣ عينة (٦٥ ٪) وتراوح تركيزها بين ٠٫٠١٠٥٩ – ٠٫٠٥٣ ملجم / كجم من الوزن الطازج, كما تم رصد وتقدير مبيد كاربيندازيم في جميع العينات التي تم فحصها (١٠٠ ٪) وتراوح تركيزه بين ٠٫٠١٤٨٥ – ٠٫٣٢٠٧ ملجم / كجم من الوزن الطازج, ايضا تم رصد وتقدير مبيد الكلوربيريفوس في ١٢ عينة (٦٠ ٪) وتراوح التركيز بين ٠٫٠٣٢٤ – ١٫٥٤٣ ملجم / كجم من الوزن الطازج , على الرغم من أن المتبقيات في معظم العينات تتجاوز الحدود القصوى للبقايا التي حددها الاتحاد الأوروبي , لم تكن هناك مخاطر صحية مرتبطة بأي من مبيدات الآفات المدروسة حيث لم تتجاوز العينات حد مؤشر المخاطر الصحية المقبول(١) و تم اختبار فاعلية الماء و بعض محاليل الغسيل في إزالة بقايا المبيدات من ثمار التمر,حيث انخفض تركيز المبيدات بشكل كبير و ازداد هذا الانخفاض بزيادة زمن الغسل ودرجة الحرارة وتركيز محلول الغسيل,اظهرت دراسة تأثير الغسل على القيمة الغذائية للتمور ان جميع المعالجات لها تأثير سلبي على القيمة الغذائية ، وازداد هذا التاثير بزيادة مدة الغسل ودرجة الحرارة وتركيز محلول الغسيل كما تم تطوير وتحسين طريقة لهضم وتقدير ثمانية معادن ثقيلة في نسيج ثمار التمر وفي الغبار الملتصق به باستخدام مقياس الطيف الضوئي ووجد ان تركيزات العناصر مقاربة للقيم المعلنة في الدراسات السابقةكما أظهر تقييم المخاطر المحسوب من متوسطات تركيز المعادن الثقيلة في العينات أن حاصل الخطر كان (<١) لجميع المعادن الثقيلة التي تم فحصها مما يشير إلى أن استهلاك هذه التمور لا يسبب مخاطر صحية للمستهلكين, أدى الغسيل بالماء لمدة ٩٠ ثانية في درجة حرارة الغرفة إلى خفض مستويات المعادن الثقيلة بشكل كبير في جميع العينات بنسب تتراوح من 0.95٪ إلى 34.78 ٪.

ABSTRACT

Toxic pollutants are a major environmental concern that has put human health at risk. Pesticides and heavy metals are at the top of the list of environmental toxins posing a threat to nature. Developing and validating methods for extracting and analyzing residual environmental pollutants such as pesticides and heavy metals in vegetables and fruits has become of great interest to ensure that the level of pollutants in them is within the permissible limits to protect the health of consumers. The acetate buffered QuEChERS (AOAC) was successfully modified and optimized for date fruit matrix extraction and pesticide residue analysis using Minitab 17.0 software and the Plackett-Burman (P-B) screening design programme. The modified method demonstrated high recovery and low matrix effect (less than 20) for the three pesticides tested, two organophosphorus (dimethoate and chlorpyrifos) and one carbamate (carbendazim). The modified method outperformed the original unbuffered method in terms of pesticide recoveries. High-performance liquid chromatography equipped with diode array detector (HPLC-DAD) method was developed and optimized for simultaneous determination of the examined pesticides in date fruit samples extracted by modified acetate buffered QuEChERS methodology. The method was validated by determining the following analytical and statistical parameters: the linearity, limit of detection limit (LOD), limit of quantification (LOQ) and accuracy of the method. The recovery (%) was found to be between 101-104.2 %. Among the examined 20 samples, dimethoate was detected in 13 samples (65%) and ranged between 0.01059 - 0.053 mg/kg of fresh weight. Carbendazim was detected in all examined twenty samples (100 %) and ranged between 0.01485 - 0.3207 mg/kg of fresh weight. Chlorpyrifos was detected in 12 samples (60 %) and ranged between 0.0324 - 1.543 mg/kg of fresh weight. The health risk assessment of the examined pesticides was calculated based on the mean concentration in each individual date fruit sample. Though the residues in most samples exceed the European Commission MRLs, there was no health risk associated with any of the studied pesticides as no samples exceeded the acceptable health hazard index (HHI) limit (<1) for adults or children. The efficiency of some wash treatments on the removal of pesticide residues from the date fruits was investigated and optimized. The methods included washing by either tap water, detergent solution and three concentration levels of either, NaCl, acetic acid, H_2O_2 or $KMnO_4$. (1 %, 2 %, and 3 % v/v) in two efferent temperature levels, 25°C and 37°C. The concentration of pesticides decreased dramatically as contact time, temperature and concentration in the fruits treated with different wash solutions. The effect of washing on date fruit’s nutrition value were studied and optimized. All treatments resulted in a negative effect on nutrition value. The decrease in nutrition value increased gradually by increasing contact time, temperature and washing solution concentration. A method for digestion and determination of eight heavy metals in date fruits flesh and in the adhered dust was developed and optimized using GFAAS spectrophotometer. The mean concentrations of heavy metals in date flesh are similar to global values and appeared to be descending in order: Fe> Al> Cu>As>Ni> Pb> Sn> Cd. The risk assessment calculated from the means of heavy metals concentration in samples showed that hazard quotients (HQs) are less than 1 for all the examined heavy metals which indicated that the consumption of these dates does not cause health risks for the consumers. The effect of water washing on various heavy metals deposited on date fruits was investigated. Washing for around 90 seconds at room temperature greatly reduced heavy metal levels in all samples. The washing effect on heavy metals removal rates ranged from 0.95 % to 34.78 %.

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LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrophotometer
DDT	Dichlorodiphenyltrichloroethane
EC	European Community
ECD	Electron Capture Detector
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
GLC	Gas Liquid Chromatography
HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexane
OCPs	Organochlorine pesticides
HMS	Heavy metals
PsR	Pesticides Residue
PSA	primary secondary amine
GPC	Gel Permeation Chromatography
ASE	Accelerated solvent extraction.
ELCD	Electrolytic conductivity detection
HECD	Hall electrolytic conductivity detection
FPD	Flame-photometric detection
NPD	Nitrogen-phosphorus specific detection
GCB	Graphitized carbon black
SPE	Solid phase extraction
MSPD	Matrix solid phase dispersion
SFE	Supercritical fluid extraction
MAE	Microwave assisted extraction
SPME	Solid phase micro extraction (SPME)
GFAAS	Graphite furnace atomic absorption spectroscopy (GFAAS)
ICP-OES	Inductively coupled plasma-optical emission spectroscopy
GAP	Good Agriculture Practice
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
PTDI	Provisional tolerable daily intake
TS	Total sugars
TP	Total phenolic
QuEChERS	Quick Easy Cheap Effective Rugged and Safe
SANCO	Directorate General Health & Consumers
S/N	Signal to Noise Ratio
SD	Standard Deviation
RSD	Relative Standard Deviation
MRL	Maximum Residue Limit
EU-MRLs	European Union Commission
HHI	Health Hazard Index
KSA	Kingdom Saudi Arabia
M.P	Melting Point
Sub.P	Sublimation Point
IPCS	International Programme on Chemical Safety
AOAC	Association of Official Agricultural Chemists (AOAC)