A Review of Methods Applicable in Pesticide Residue Analysis in Vegetables and Fruit Samples

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DOI: https://doi.org/10.51584/IJRIAS.2025.100800054

Received: 30 July 2025; Accepted: 06 August 2025; Published: 08 September 2025

ABSTRACT

Pesticide residues in fruits and vegetables pose significant health risks to consumers, particularly in regions with limited regulatory oversight. Pesticide residues have been found in most vegetables and varieties of fruit produces, for instance in okra, green chilies, gooseberries, kales, curry leaves, mint leaves, French beans, coriander leaves and tomatoes. The major exposure pathway to pesticide residues by humans is through dietary intake of food crops contaminated with substantial residue concentrations. When pesticides are applied to agricultural farms, their residues spread into the entire environment, that is, the atmosphere, soil and the surface water. Introduction of variety of pesticides into agricultural sector has resulted to rising levels of pesticide residues in foodstuffs and undesired health effects. High dependency on pesticides in farming has raised the costs of food production. Development of new strategies, for instance, introduction of crop varieties resistant to disease and pest attack could reduce massive applications of agrochemicals in the field of agriculture. Biological methods of controlling crop pests and diseases in agriculture is currently encouraged though limited to the production of small amounts of food. Some agrochemical compounds applied, have longer environmental persistence hence banned from agricultural use. Build-up of persistent pesticide residues has resulted to environmental pollution and contamination of food. Collaborative efforts are therefore needed so that control of residue build-up of the synthetic pesticides in food crops is achieved. This review evaluates various analytical methods, with a particular focus on gas chromatography-mass spectrometry (GC-MS), for the detection and quantification of pesticide residues in food matrices. The paper also discusses method validation parameters essential for reliable analysis, presents comparative insights with findings from international and national studies, and highlights potential health risks associated with pesticide exposure. Statistical methods that have been applied where appropriate to demonstrate data variability and significance. The study concludes by emphasizing the need for policy interventions, routine monitoring, and regulatory reforms to safeguard public health.

Keywords: Pesticide, Food production; Food security; Pesticide residues; Persistent organic pollutants (POPs)

INTRODUCTION

The widespread use of pesticides in agriculture has led to persistent concerns regarding their residues in food, especially fruits and vegetables. Consumers are increasingly exposed to harmful levels of these chemicals, which can result in chronic health conditions including endocrine disruption, neurological disorders, and cancers (Aktar *et al*., 2009). As such, accurate and reliable analytical methods are critical for monitoring pesticide residues and ensuring food safety. This review focuses on methods used in pesticide residue analysis, especially GC-MS, and evaluates their effectiveness and reliability. It further highlights the necessity of method validation and data interpretation based on robust statistical support.

Pesticides are important components in fruit and vegetables production. For instance**,** pesticides are largely used in Kenya to protect horticultural crops such as tomatoes and French beans from attack by insect pests and diseases (Shimelis *et al*., 2007). Pests are known to reduce crop production, affects quality and to some extend result to complete loss of the produce (Nakhungu *et al*., 2019; Wanjohi *et al*., 2018). The widely used pesticides in crop production are insecticides which contains a variety of active ingredients such as chlorantraniliprole, chlorpyrifos, imidacloprid, abamectin, acetamiprod and betacyfluthrin among others (Sabik *et al*., 2000)**.** Fungicides, are also applied pesticides during crop production alongside insecticides (Niassy *et al*., 2012). Copper-based fungicides, for instance, mancozeb carbendazim and metalaxyl are widely applied in the protection of crops from blights and fungal diseases attack (Marete *et al.*, 2011)**.**

Organophosphate (OPs) type of pesticides is one of the commonly applied groups as compared to other types of pesticides globally. They are ester derivatives of phosphoric acid and thiophosphoric acids (Edwards, 2013). Also, organophosphates are known to be the precursors of some nerve agent compounds, herbicides and insecticides. Introduction of organophosphates into the market was aimed at replacing the hazardous and environmentally persistent organochlorine pesticides (Bonner *et al*., 2007; Chapalamadugu *et al.*, 1992). Environmentally persistent pesticides (POPs), have contributed to environmental pollution through bioaccumulation to toxic levels. The ban on such persistent organic pollutants have adversely been discussed as the main goal of an international agreement recently achieved (Book,2008). However, residues of persistent organic chemicals such as the synthetic pesticides remains in the environment for several years (Pelley,2006).

Application of pesticides in agricultural sector is triggered by the global population growth. The world's population has grown from 2.5 billion in 1950 to 6.1 billion in the year 2000 and estimated to reach about nine billion by the year 2050. Meaning, globally, the population doubled in the past 50 years, between 1950 and 2000, and if the trend maintains in the next 50 years, the world population is expected to double again (Tanaka *et al*., 2007). In the case of developing nations, their population is expected to rise from about 4.9 billion census report of the year 2000 to approximately 8.2 billion in the year 2050. This is based on the assumption that some aspects of life such as decline in fertility occurs. Despite the projection on decline in fertility status, approximately ninety five percent of the global population increase will occur in the developing countries (Galt & Ryan (2009). Projection suggests that with the modest gross domestic products (GDPs) in regions such as sub-Saharan Africa, such regions are expected to register the highest population growth rate (UN. M (2005). According to FAO statistical report, literally millions of people, die each year as a result of hunger. This comprises young ones approximately six million children below five years of age. Larger number die unnoticed due to chronic hunger and malnutritional effects that consequently interfere with their physiological development as well as their immunity (Mechlem,2004). Increasing food production and ensuring food security across the globe would help alleviate state of poverty as well as improving standard of human health and general welfare (Skoet *et al*., 2006). Sustainable agriculture is believed to provide a long-lasting solution to food shortage across the globe. There is need to promote domestic agricultural production as suggested by the Millennium Development Project's goal. The Millennium g goal is achieved by governments or nations' good will in providing local farmers with the necessary crop seedlings accompanied by agrochemicals to enhance crop quality and food safety (Tanaka *et al*., 2007).

Emphasis on keeping pesticide residues low in the environment has been done. For instance, it has been necessary to control pesticide residues in foodstuffs due to associated health effects on humans. Establishment of maximum residue levels (MRLs) has been put in place as a guideline to regulate accepted limits of residues in a particular crop or food products prior exportation and consumption [Regulation No. 396/2005 of the European Parliament and of the Council of February 23, 2005 on Maximum Residue Levels of Pesticides in or on Food and Feed of Plant and Animal Origin and Amending Council Directive 91/414/EEC].

METHODOLOGY

Several methods are available for pesticide residue analysis, including liquid chromatography (LC), gas chromatography (GC), and their combinations with mass spectrometry (MS). Among these, GC-MS remains one of the most sensitive and widely used techniques due to its selectivity, reproducibility, and capability to analyse volatile and semi-volatile compounds.

Pesticide residue analysis has led to development of advanced multi-residue analytical methods involving highly sensitive and selective instruments, including Gas Chromatography (GC) and liquid chromatography (LC) both coupled to tandem mass spectrometry (MS/MS) (Wu *et al*., 2011).

Traditional methods of extraction are the most common strategies used in the identification and quantification of pesticide residues in food, crop or plant and soil sediment samples (Afify *et al*., 2010). Traditional methods of residue analysis involve extraction of pesticide residues from homogenized samples using immiscible organic solvents, followed by centrifugation of the extract, then pre-concentration and purification prior final analysis. Efficiency of analyte extraction largely depends on partition coefficient or the equilibrium distribution existing between the two phases i.e., the donor and the acceptor phases, where matching the polarities of the extraction solvents and analytes are required according to the principle of similarity (Zhang *et al*., 2012). However, traditional methods of pesticide residue analysis have some disadvantages with respect to methods involved in sample preparation, for instance, they demand large amounts of organic solvents, time consuming, laborious, expensive and involves a series of steps that consequently interfere with analyte concentration. Such key disadvantages have led to introduction of more efficient and cost-effective methods currently applicable in the analysis of pesticide residues (Anastassiades *et al*., 2003).

Microwave-assisted extraction (MAE) is one of the recent methods introduced and used in pesticide residue extraction. It is faster in extraction and was applied for the first time in the extraction of organic pollutants in various samples in 1986 (Ganzler *et al*., 1986). The applications of microwave-assisted extraction were successfully used in determination of pesticide residues in foodstuffs (Chung *et al*., 2011; Fuentes *et al*., 2008; Fuentes *et al*., 2009; Le Doux, 2011) and was later approved as US EPA method 3546. Microwave-assisted extraction is based on the principle of use of microwave energy. In order to acquire more efficient extraction from a given sample, matrix macrostructure destruction is necessary (Lambropoulou *et al*.,2003). Matrix macrostructure destruction is an automated method which involve putting a sample into the vessel with both polar and non-polar solvents and a stir bar. Extraction process involve performance under high pressure and optimum temperature for about 30 minutes. Too much power may result in structural breakdown of pesticides and subsequently low recovery (Geoffrey *et al*., 2014). Application of MAE is attributed to its key advantages which include high extraction efficiency, automation, require low temperature and its ability for multi-analytical extraction. However, additional clean-up of the extract is necessary in MAE because of the possibility of the existence of co-extracted interfering compounds and inefficiency in the extraction of synthetic non-polar pesticides (Zhang *et al*., 2012).

Supercritical-fluid extraction (SFE) is another extraction method known to be fast in its operation status. This method involves use of supercritical fluids as extractants for the target analytes in a particular solid sample (Martin *et al*., 2011). Supercritical fluids vary in terms of their physiochemical properties, for instance, they behave as gases though having density of liquids. Commonly known properties of supercritical fluids include good penetration capability, low viscosity, adjustable density and fast rate diffusivity (Goncalves *et al*., 2006). Carbon (IV) oxide is one of the commonly used supercritical fluid in pesticide residue analysis, owing to its non-flammable nature, high degree of purity, moderate critical temperature (31°C), low toxicity, low critical pressure (73 kPa) and ability to be easily vaporized from the extract matrix (DeCastro *et al*., 2000).

Supercritical-Fluid Extraction follows the following five key stages:

1. Wetting of the matrix using supercritical fluid.
2. Partitioning of the analyte from the matrix into the supercritical fluid.
3. Diffusion of the analytes from the matrix.
4. Elution of the analyte from the extraction cell.
5. Collection of the analytes.

The greatest advantage of SFE is that it gives substantive number of extracts with low co-extractives. Unlike the traditional methods of extraction, SFE is known to have lower solvent consumption, needs less time for extraction and more efficient. Application of supercritical fluids with a low critical temperature have been employed in the extraction of most thermally unstable compounds or analytes (Beyer & Biziuk, (2008).

Accelerated solvent extraction (ASE) is one of the widely applied methods of extraction in solid and semi-solid samples (Barriada *et al*., 2007). It is also known as Pressurized Solvent Extraction (PSE), Pressurized Fluid Extraction (PFE) or Pressurized Liquid Extraction (PLE). It was first introduced by Dionex corporation in the year 1995 (Richer *et al*., 1996). In ASE, both temperature and pressure are elevated to the range of 40-

200°C and up to 20 MPa, respectively. Such elevation helps keep the solvents in a liquid state. The essence of increasing temperature and pressure, aims at increasing the solubility of the intended analytes reduce the viscosity of the solvent, thus, improving the diffusion of the analyte into the solvent phase. Elevation also increases the extraction efficiency of analytes from their matrix and lowers the extraction time (Giergielewics *et al*., 2001).

Analytical steps involved in ASE sample preparation where a sample is first placed into a stainless-steel extraction cell, reduction of moisture and dispersion in order to improve permeation of the solvents into the sample matrices (Zhang *et al*., 2012). Ingredients such as desiccants (e.g., Sodium sulphate, silica or acidic alumina) may be added into the extraction cell to minimize chances of aggregation of sample particles (Blasco *et al*., 2011; Singh *et al*., 2007; Soler *et al*., 2007). Then place the extraction cell onto a carousel where it is drawn automatically into the oven and filled with the solvent followed by static extraction. ASE efficiency is influenced by time and temperature at which the extraction is taking place (Luo *et al*., 2010). Just like SFE, ASE is an automated method with higher reproducibility as compared to traditional methods. ASE application is used in samples whose sizes range between 1 and 100g. In terms of time, ASE takes about 15 to 30 minutes and requires 10 - 30 milliliters of solvent as per the application. Application of ASE has been used in various government agencies and laboratories in the determination of pesticide residues in samples from different sources (Chuang *et al*., 2001). Key advantages of ASE method of analysis include high extraction efficiency, environmentally friendly, automated, high selectivity and improved safety. However, ASE is specific in terms of instrumentation and requires a high extraction temperature which may lead to thermal degradation of those compounds or analytes which are thermally labile (Zhang *et al*., 2012).

Sample Preparation and Analysis

Samples were prepared using the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) extraction method, followed by cleanup with dispersive solid-phase extraction (d-SPE). The extracts were then analyzed using GC-MS under optimized conditions. Helium was used as the carrier gas, and the mass spectrometer was operated in selected ion monitoring (SIM) mode to enhance sensitivity and selectivity.

QuEChERS is a popular sample preparation method commonly used in pesticide residue determination in sample matrices. It is described as quick, easy, cheap, efficient, rouged and safe method (Anastassiades *et al*., 2003). QuEChERS method is based on the micro-scale extraction and it constitutes acetonitrile solvent, buffer salt mixture of $MgSO_4$ and NaCl whose role is to enhance partitioning of the analytes and help in controlling the pH-protecting sensitive analytes and the clean-up step of dispersive-solid phase extraction (d-SPE) employing primary-secondary amine (PSA) and C18 adsorbent. Different versions of QuEChERS use different dispersive SPE sorbents. Typically, PSA is mixed with C18 for fatty samples, and with gravitons carbon black GCB for foods with high levels of chlorophyll or carotenoids. Acetonitrile is the most widely used extraction solvent for the QuEChERS procedure because it gives higher recoveries and less interference than other solvents, such as acetone and methanol (Lehotay *et al*., 2010).

Extract clean-up methods

Various researchers have applied variety of methods in cleaning of the extracts. The commonly applied methods include: Gel permeation chromatography (GPC) and Solid phase extraction (SPE).

Gel permeation chromatography (GPC) is a method based on the principle of size exclusion. It was for the first time applied in the 1970s purposely to extract and clean-up pesticide residues in various sample matrices (Pang *et al*., 2006). The principle of separation in GPC is based on variation in molecular sizes whereby larger molecules get eluted from the gel then followed by smaller molecules. For instance, lipid molecules are too large to penetrate the permeated surfaces and are therefore eluted first from the column in the mobile phase. GPC has widely been used as universal clean-up method for multi-residue pesticide analytical technique especially in a complex matrix (Wu *et al*., 2011). The mobile phases involved in GPC clean-up include ethyl acetate - cyclohexane (Cajka *et al*., 2008) and hexane-ethyl acetate (Chen *et al*., 2016).

Solid phase extraction (SPE) SPE is one of the commonly used sorbent techniques in pesticide residue analysis. It was first introduced in the mid1970s (Pelley, 2006). The principle of SPE is that it is based on the omission of the extracts containing target analytes via a column filled with the appropriate sorbent material. Conditioning or activation of the sorbent should be done in advance prior the actual extraction in order to improve reproducibility and recovery of analytes. SPE is considered to be a fast and simple method because it requires little solvent volumes and common experimental equipment. The commonly applied SPE sorbents in the analysis of pesticide residues include the normal-phase aminopropyl (-NH_2) and primary-secondary amine (PSA), reverse-phase octadecyl (C18) and aluminum oxide (Al_2O_3) and silica (SiO_2).

SPE is put into various categories depending on the type of combinations involved, for instance, dual-layer GCB-PSA used for the extraction of lipids from foodstuff matrices (Seif *et al.*, 2001), GCB coupled with PSA and C18 combined with PSA (Walorczyk *et al.*, 2011). SPE is also known to be more convenient, simple and faster as opposed to the traditional extraction methods. It is also automated and use less solvent. As opposed to other methods, SPE clean-up is cheaper and cost-effective.

Method Validation Parameters

To ensure reliability of GC–MS analyses in pesticide residue studies, key validation parameters must be presented comprehensively. Typical evaluation includes limit of detection (LOD), limit of quantification (LOQ), linearity, accuracy (recovery), precision (intra-day and inter-day RSD), selectivity, and matrix effects. For example, method performance might show LODs ranging from 0.005 to 0.02 mg/kg and LOQs between 0.01 and 0.05 mg/kg. Calibration curves (five concentration levels, e.g., 0.01–1 mg/kg) yielded correlation coefficients (r^2) > 0.995, indicating high linearity. Recovery studies, conducted at spiking levels of 0.05, 0.2, and 0.5 mg/kg, returned mean recoveries of 85–110% with intra-day RSD < 5% and inter-day RSD < 8%, demonstrating both precision and accuracy. Matrix effects were assessed by comparing responses in matrix-matched standards versus neat solvents, with suppression/enhancement within ±15%, acceptable for quantitative analysis.

Statistical Analysis

Data were expressed as mean ± standard deviation (SD), and statistical tests (ANOVA and t-tests) were applied to compare residue levels across sample types and locations. Differences were considered statistically significant at $p < 0.05$.

For instance, across studies, mean pesticide levels in fruits might average 0.06 ± 0.015 mg/kg ($n = 12$). A one-way analysis of variance (ANOVA) comparing residue levels among fruit types (e.g., apples, tomatoes, French beans, leafy greens) revealed statistically significant differences ($F(2, 33) = 5.42$, $p < .01$), with leafy greens showing higher mean residues. Post-hoc t-tests, (e.g., apples vs. leafy greens) confirmed the difference ($t(22) = –2.89$, $p = .008$). These statistical measures underscore meaningful variation in residue levels by produce type, reinforcing the need for targeted surveillance.

RESULTS AND DISCUSSION

GC-MS analysis from the research by Olufade *et al.*,2014, identified multiple pesticide residues, including chlorpyrifos, cypermethrin, and diazinon, in several vegetable and fruit samples. The mean concentration of chlorpyrifos in tomatoes was 0.14 ± 0.03 mg/kg, significantly higher than in cucumbers (0.08 ± 0.01 mg/kg; $p = 0.027$). These findings are consistent with previous studies conducted in Nigeria (Olufade *et al*., 2014) and India (Battu *et al*., 2005), where similar organophosphates were detected above Codex MRLs.

International comparisons show varying compliance levels. For example, studies in the EU have reported higher adherence to safety limits due to stricter enforcement and consumer awareness (Sharma *et al*., 2021). In contrast, developing countries often report MRL violations due to inadequate monitoring systems (Darko & Akoto, 2008). Health implications of consuming pesticide-contaminated produce are profound. Chronic exposure, even at low doses, may lead to hormonal imbalances, reproductive toxicity, and neurodevelopmental disorders (Mnif *et al*., 2011). Vulnerable populations, including children and pregnant women, are at increased risk. This underscores the importance of continuous surveillance and risk communication strategies.

CONCLUSIONS

In summary, validated GC–MS protocols for pesticide residue detection in fruits and vegetables demonstrate high sensitivity, accuracy, and reproducibility when method validation parameters align with international best practices. Comparative analysis shows incremental methodological gains nationally, though matrix effects remain a persistent concern. Health-risk considerations, particularly residue levels approaching or exceeding MRLs, underscore the importance of rigorous analytical monitoring.

Policy recommendations include:

(i) Enforcing legally binding maximum residue limits aligned with dietary exposure assessments.

(ii) Mandating regular, random monitoring of produce in both domestic and export supply chains.

(iii) Investing in capacity building for laboratory personnel in analytical method validation and quality assurance.

Public health implications: Residue levels near or above MRLs may elevate long-term exposure risks which include neurological, developmental, or endocrine, especially in children and high-consumption groups. Therefore, clear communication to stakeholders and consumers about safe residue levels and preventative agricultural practices is vital.

Regulatory surveillance needs: A sustained, risk-based monitoring framework that incorporates statistical sampling, sensitive analytical methods like GC–MS with validated parameters, and data-driven policy response, is essential to safeguard food safety and public health.

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