Advancing the Innovative Approaches of Detecting Pesticidal Residues in Vegetables: A Review of Technological Innovations

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Abstract

The growing demands for food with ever increasing human population have widespread use of pesticides. Pesticide residues may possess short-term or long-term toxicity issues depending upon their type and exposure. Pesticides can have a variety of detrimental effects on their anticipated target, which includes humans. Therefore, there is great need to firmly regulated and monitored properly pesticide residues in foods to protect the healthiness of consumers. To do this, precise, responsive, and robust investigative methods are essential for effective management of pesticide. Liquid chromatography-tandem mass spectrometry and gas chromatography-tandem mass spectrometry techniques are often used. The most popular method for detecting pesticide residues is to utilise a triple quadrupole mass analyzer in the selective reaction monitoring mode. However, at least two products are required for chemical identification, and the ion ratio from sample extracts should be within 30 % of calibration standards from the same sequence. This belief encompasses several technologies aimed at achieving speedy, selective, cost-effective, and sensitive food safety screening. Optical or electrochemical transducers are frequently used to monitor biorecognition events, which are primarily based on bio-affinity interactions between selective biomolecules such as antibodies or enzymes and pesticide residues. Therefore, the present review is aimed at discussing the various rapid detection methods to detect the presence of pesticide residues in vegetables and foods.

Key words: Pesticides, detection methods, techniques, vegetable foods, toxicity

1. Introduction

The increasing demand for food production entailed the widespread use of pesticides. According to the "European Commission," pesticides may prevent, eliminate, control pests and diseases, or protect plants from pests and other plant products during manufacturing, storage, and transportation. These pesticides can be classified into many types depending on the target pest; for example, chemicals that kill or repel insects are known as insecticides [1]. The World Health Organization has also advised categorising based on the threat displayed as fatal dosage in a rat model (World Health Organization, 2009). On the other hand, pesticides can be classified based on their mechanism, such as how they interact with the target pest [2]. Pesticide residues are associated with short-term or long-term toxicity issues. Pesticides can have a variety of effects on their intended target, which includes humans and animals. The neurological system has been found to be stimulated by organo chlorine insecticides. Throughout the twentieth century, organo chlorine pesticides were commonly used. For instance, Linden inhibits the influx of calcium ions and the Ca or Mg ATPase pump, resulting in the release of neurotransmitters [3]. It also acts as a hormone-destroying agent, causing short-term and long-term side effects such as skin inflammation, headaches, cancer, Parkinson's disease, and immune system imbalances [4]. Carbamate (CM) and organo phosphorus (Ops) pesticides act to inhibit acetylcholine esterase (AChE). Acetylcholine esterase is a very important enzyme found in the nervous systems of mammals and insects. In general, acetylcholine esterase causes the hydrolysis of acetylcholine to choline and acetic acid. This response is very important because it facilitates the return of cholinergic neurons to their inactive state after activation. However, in the presence of CM and organo phosphorus, "carbamylation" or "phosphorylation" of the hydroxyl group of serine at the enzyme's active site reduces AChE activity [5]. As a result, acetylcholine accumulates and can cause serious health problems such as respiratory and heart dysfunction [6]. Therefore, it is clear that pesticide residues in foods need to be firmly regulated and monitored properly to protect the healthiness of consumers' especially human beings.

To do this, precise, responsive, and robust investigative methods are essential for effective management of pesticide remains in food media. Modern quantification techniques such as "Liquid chromatography-tandem mass spectrometry (LCMS/MS)" and "Gas chromatography-tandem mass spectrometry (GC-MS/MS)" techniques are often employed [7,8] in several samples, e.g., vegetables and fruits [9], honey [10]; rice [11]; and food of animal origin [12]. This allows for a wide linear range and detection limit (LOD) up to the µg kg^{-1} level. The most popular method for detecting pesticide residues is to utilise a triple "quadrupole (QqQ) mass analyzer" in the selective reaction monitoring (SRM) mode. However, at least two products are required for chemical identification, and the ion ratio from sample extracts should be within 30 % of calibration standards from the same sequence. As a result, this need emphasises a significant disadvantage of SRM mode: the greater the number of pesticides used in the approach, the more ion transitions must be monitored. Thus, there's a higher probability that frequent or overlapping transitions will influence the method's detectability [13]. In this context, "High-resolution mass spectrometry (HRMS)" methods with targeted resolution have been envisaged [14–16]. Mass detectors such as Orbitrap, Time of flight (TOF), and hybrid analyzers have an accurate mass measurement (5 ppm), higher resolution (full width at half maximum (FWHM) 20,000 or more), and provide structural explanation and full MS scan-feature (usually in the range of 100-

1000Da). The HRMS detector eliminates SRM-related issues, but the quantification capabilities compared to the QqQ approach are still being discussed. In this way, screening approaches for food contaminant analysis have been created, and they have a lot of potential [8]. "Screening procedures are used to identify the presence of a chemical or class of compounds at the level of interest," according to Decision 2002/657/EC. This notion encompasses several technologies aimed at achieving speedy, selective, cost-effective, and sensitive food safety screening [17]. Optical or electrochemical transducers are frequently used to monitor biorecognition events, which are primarily based on bio-affinity interactions between selective biomolecules such as antibodies [18] or enzymes [19] and pesticide residues [20]. Optical conversion systems correlate biometric events with colour development and change, suggesting their use. Combining this optical screening approach with smart phones can increase the potential for ubiquitous biosensing [21]. Smart phones have brought the following anomalous aspects to chemical analysis: online results or "end-user implementation". This can also affect the analysis of pesticide residues [22]. Therefore, the present review is aimed at discussing the various rapid detection methods to detect the presence of pesticide residues in vegetables and foods.

2. Classification and toxicity of pesticides

Pesticides are classified based on their chemistry, source, and intended target organism. They can be inorganic, synthetic (manufactured synthetically) or biological (bio-pesticide). Insecticides, herbicides, fungicides, rodenticides, and nematicides are the most common types of pesticides **(Table 1)**. Synthetic inorganic pesticides are insecticides of artificial origin that kill bugs directly. In the past few decades, the bio-pesticides derived from natural resources such as plants and microbes have gained much research interest. Furthermore, insecticides are categorised into four main classes: (i) organochlorines (OCs), (ii) organophosphates (OPs), (iii) carbamates, and (iv) pyrethroids **(Table 1)** [23]. OC pesticides are mainly used pesticides and have significant toxicity and *in vivo* accumulation [24]. They are also cancer-causing, estrogenic, and resistant to the decomposition cycle in the environment, with a half-life of 11-31 years [25]. In Europe, America, and several Asian nations, OCs has been prohibited and is no longer used for agricultural reasons. OCs has been phased out, but their persistence has made them clearer in the environment and they have been replaced by other artificial compounds such as OP and carbamate. Because of their low cost, low persistence in environmental situations, and potential to kill a wide range of pests, OP and carbamate insecticides are presently frequently used. Upon assimilation, OPs and carbamates may disturb normal CNS function by blocking the enzyme acetylcholine esterase in humans and insects [26]. According to an estimate, more than 80 % of pesticide-related hospitalizations are due to OP chemicals [27]. Although, as compared to OCs, their harmful consequences are less severe [28]. Likewise, pyrethroids are insecticides manufactured from natural "chrysanthemum ester-containing" ordinary substances known as "pyrethrins", as opposed to synthetic pesticides [29]. Artificial pyrethroids have a long half-life and are more stable in the environment than natural pyrethroids. Their unique insecticidal activity, as well as their low toxicity to mammals, has piqued their interest. They act by delaying the "voltage-gated sodium channel" in the membrane of the neuron [30]. Regardless of the pesticides used to manage pests in agriculture, their accumulation as residues in food is harmful to both human beings and environment [31,32]. When these pesticide

remains are taken up, they build up in the tissues of human beings, leading to weakness of muscles, endocrine abnormalities, diseases of the respiratory system, paralysis, cancer, and other health problems [33,34]. As a result, considering the importance of these residues in supporting plant growth, considerable steps should be taken to check their presence in food items. Previously, different ways were identified to detect the pesticides residues from various vegetable and fruits.

Table 1 Classification and categorization of pesticides

Category of Pesticide	Classes of Pesticides	Mode of Entry	Targets	Examples
Insecticide	Organophosphates, Carbamates, Pyrethroides, Organochlorines	Systemic, Non-systemic, Stomach Poisoning, Fumigants, Repellants	Used for insect control.	Malathion, Carbaryl, Cypermethrin, Lindane
Herbicides	Phosphonates, Chlorophenoxy herbicides, Dipyridyl herbicides		Used to destroy weeds or prevent their growth	Glyphosate 2,4-D
Rodenticides	Anticoagulants, Convulsants, Metabolic poison		These are used to kill rodents.	Warfarin, Brodifacoum Strychnine
Fungicides	Thiocarbamates, Triazoles, Strobilurines		Used to destroy fungi, since many plant diseases are caused by fungi.	Fluconazole, Triadimefon, Metam-sodium
Fumigants	Halogenated Organic, Inorganic		Used to destroy nematodes, insects	Methyl bromide, Chloropicrin, Carbon disulfide,
Miscellaneous	Arsenicals, Pyridine			Lead arsenate, Chromated copper arsenate

3. Detection of pesticide: Conventional and Modern Approaches

The detection techniques for pesticides are broadly categorized under groups namely, (i) Pre-treatment and extraction methods, (ii) Detection method using instruments, and (iii) Optical screening methods **(Fig. 1)**.

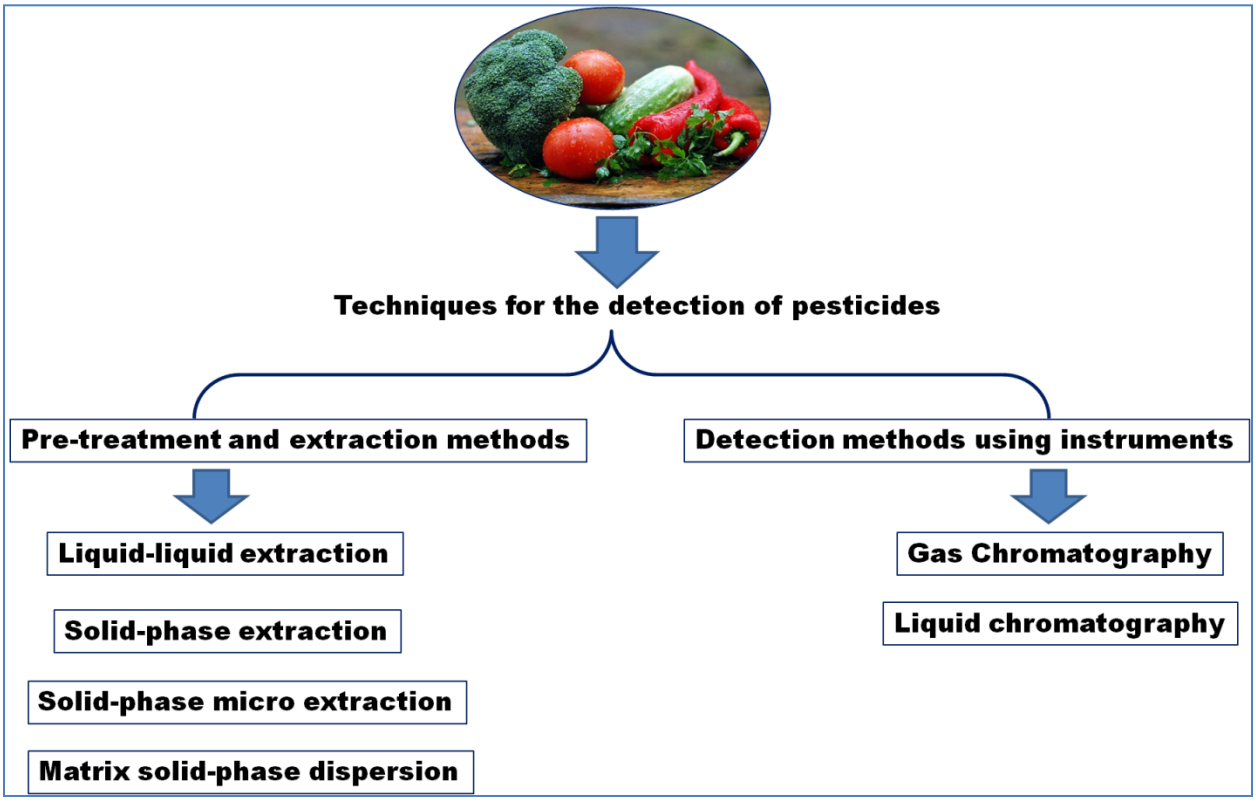

Fig. 1 Various pre-treatment and extraction methods for the detection of pesticides in vegetable foods.

4. Pre-treatment and extraction methods

The residues of pesticides in vegetable foods are determined using various pre-treatment and extraction procedures. There are no common, conventional, or standard pesticide removal processes in laboratories. Furthermore, the removal procedure is a pathway that requires the extraction of the target analyte from the medium, followed by a cleanup step or set of steps in the analytical method in which the majority of potential intervention co-extracts are eliminated using physical or chemical means (solid-liquid or liquid-liquid extraction). The production of subsamples is the first step in the extraction technique. After cleaning, the starting material comprises 0.5-2 kg of samples that are homogenised using a mixer. Homogenized sub-samples weighing between 0.5 and 100 g are obtained for additional extraction. According to the literature, "acetonitrile, ethyl acetate, dichloromethane, methanol, and toluene" are a few of the popular solvents investigated for determining pesticide residue in vegetable foods. Likewise, various solvent mixtures were also employed in some cases to improve technique recovery. Furthermore, agents like NaOH and CH_3COOH are used to neutralize the medium, resulting in improved recovery [35]. Due to their ease of use, "liquid-liquid extraction (LLE)" and "solid-phase extraction (SPE)" were initially used in the extraction process. In the past decade, "micro-scale extraction process", have gained much research attention due to their quick, easy, cheap, effective, rugged, and safe (QuEChERS) characteristics [36]. The extraction of organic compounds from various matrices is a lengthy process; however, the quick, easy, cheap, effective, rugged, and safe method decreases analysis time, decreases the number of analysis stages by employing a few reagents in small quantity, and improves recovery. Afify et al. (2012) compared to the quick, easy, cheap, effective, rugged, and safe, ethyl acetate, with conventional "Luke extraction" methods [37,38].

According to the data, the Luke method had a substantial impact on the recovery of non-polar and medium-polar compounds, whereas ethyl ethanoate alone resulted in significant polar chemical recovery. Throughout a recovery range of 60-70 %, for non-polar and medium-polar compounds, the QuEChERS approach showed good recovery. However, when compared to the other ways, Luke's cleaning approach was judged to be the simplest. Still, the extraction method has gotten better over the last few years, making sample preparation easier, shortening the time it takes to analyze, and getting rid of the use of harmful solvents.

4.1. Liquid-liquid extraction (LLE)

The separation of substances in extraordinary immiscible liquids, principally based on their respective solubilities, is known as LLE or solvent extraction and partitioning. It is a reliable, adjustable, and compatible extraction method with a wide range of devices. Liquid-liquid extraction is one of the most well-known and well-established pesticide extraction processes. The commonly used medium polarity solvents in liquid-liquid extraction for the extraction of pesticides from food medium are C_6H_{14}, methyl cyanide, and ethyl ethanoate **(Table 2)** [39]. In addition to the aforementioned solvents, Wang et al. (2012) employed the LLE approach using a chloroform/dichloromethane combination to determine seven neonicotinoid insecticides (nitenpyram, dinotefuran, clothianidin, thiamethoxam, acetamiprid, imidacloprid, and thiacloprid) [40]. The method's detection limit was obtained throughout a range of 0.002-0.005 mg/kg, with a correlation value (R2) of 0.99, and recovery was 76-123 %. Similarly, Grimalt et al. (2010) used another LLE approach to identify eleven pesticides from several classes at the same time [41]. Methanol-water extraction was performed and filtered using "nylon syringe filters" before analysis. Even though LLE techniques are widely used, they have several disadvantages, including the use of significant amounts of hazardous solvents, a long analysis time, difficulty automating, and a lack of effectiveness against polar molecules. Also, because the LLE method doesn't distinguish between analytes, it pulls all molecules out of the matrix, which causes a lot of interference from the matrix.

Table 2 Extraction and detection of pesticides employing LLE techniques from various vegetables and fruits foods

Sample	**Solvent**	**Limits of Detection**	**Limits of Quantification**	**Pesticide recovery (%)**	**References**
Fruits and vegetables	Acetone and dichloromethane	0.006-0.015 mg/kg	0.02-0.05 mg/kg	77.1-98.7	[42]
Apricot juice, apple juice, and grapes juice	Secondary amine and carboxylic acid	0.32-0.51 ng/ml	1.09-1.72 ng/ml	69-91	[43]
Tomato, peach, grape, and apricot	Methyl chloroform	0.21-0.34 µg/L	0.69-1.12 µg/L	49-70	[44]
Tomato and cucumber	Chloroform	0.5-1.0 ng/g	5.0-300 ng/g	84.6-97.5	[45]

Soybean oil	Acetonitrile and petroleum ether	0.004-0.05 mg/kg	-	80-114	[46]
Apple, tomato and grape	Glycerol-proline	0.05-0.2 µg/ml	0.1-0.5 µg/ml	87.5-113	[47]
Spinach	Acetonitrile	≤ 0.003 mg/L	0.0002–0.01 mg/kg	70-120	[48]
Coconut water	Hexane-dichloromethane (1:1, v/v)	0.002-2.0 mg/kg	0.01-7.0 mg/kg	75-104	[49]
Honey	Acetonitrile: ethyl acetate (6.5 mL:1.5 mL)	0.016 µg/g	0.032 µg/g	85.0	[50]
Edible blended oil s	Acetonitrile	0.002-0.04 mg/kg	0.0006-0.0012 mg/kg	74.0	[51]
Honey	Acetonitrile	0.33-1.97 ng/g	1.48-23.65 ng/g	34-96	[52]

4.2. Solid-phase extraction (SPE)

Because of its simplicity, speed, and capacity to handle a large number of samples with good recovery, SPE is the most frequently used approach. A variety of solid-phase extraction cartridges are used for pre-treatment and testing of pesticide remains in vegetable foods **(Table 3)**. The florist column, C18 columns, and Envicarb cartridges have all been used to assess pesticide residues. For assessing neonicotinoid pesticides, Liu et al. (2010) devised an extraction technique using a solid phase extraction HLB cartridge and an "Extrelut NT 20 column" [53]. Balinova et al. (2007) devised an extraction technique based on sorbents with various retention mechanisms (SAX, PAX, and GCB sorbents). Graphitized carbon black (GCB) and primary secondary amine (PSA) are dispersive SPE sorbents used to estimate residuals in the majority of cases [54]. To boost the sensitivity of the procedures, graphitized carbon black (GCB) and primary secondary amine (PSA) sorbents are sometimes utilised in combination in the cleaning procedure. "Multi-walled carbon nano tubes (MWCNTs)" are used in pesticide extraction in addition to the sorbents mentioned above because of their efficacy. In a study by Fan et al. (2014), MWCNTs were used as an SPE sorbent to remove 36 residues of pesticides from spinach and cauliflower [55]. The MWCNTs may perform better with high polar insecticides, according to the findings. Organic solvents, in addition to sorbents, have been used to extract and elute pesticide residues in solitary or mixed forms, making it critical to choose the proper solvent for the job. The solvents utilized are determined by the pesticide's molecular properties (ionic and non-ionic). For pesticide analysis, the SPE technique is said to be the fastest and most efficient. Even though SPE approaches enable greater separation and recovery from complicated matrices, mastering their application is a time-consuming task. Furthermore, because of the interaction of sorbents with analytes, this approach might clog cartridges due to suspended materials in the samples, and thus has the potential for poor recovery.

Table 3 Detection of various pesticides from vegetables and fruits foods using solid-phase extraction methods

Sample	Cartridge	Limits of Detection	Limits of Quantification	Pesticide recovery (%)	References
Soybean oil, sunflower oil, and extra-virgin olive oil	EMR-Lipid sorbent with Z-Sep and PSA	10.2-156.0 μg/kg	10 μg/kg	70-120	[56]
Corn oil, blend oil, soybean oil, rapeseed oil, and peanut oil	UiO-66 with uniform micropores	0.16-1.56 ng/g	0.61-5.00 ng/g	81.1-113.5	[57]
Cucumber	Molecularly imprinted polymers formed by 4-(dimethoxyphosphorothioylamino)butanoic acid	8.73-19.78 μg/kg	-	82.5-95.6	[58]
Onion and tomato	Polysulfone magnetic nanoparticles	0.28-0.54 μg/L	0.96-1.9 μg/L	46-95	[59]
Cabbage, rape, Chinese kale, amaranth, broccoli, and cauliflower	Polystyrene-coated magnetic nanoparticles	0.0200-0.0392 ng/g	0.072-0.128 ng/g	91.6-116.2	[60]
Cucumber, leek, and pakchoi	Molecularly imprinted polymer was synthesized using O,O-dimethyl thiophosphoryl chloride	0.13-0.90 μg/kg	0.43-2.97 μg/kg	80.11-97.70	[61]
Strawberries, tomatoes, cucumbers and cabbages	Polyaniline-modified zeolite NaY	0.001-1.00 mg/L	0.005-2.50 mg/L	64-128	[62]
Benincasahispida and pakchoi	Graphene oxide-based composite	0.3-0.5 ng/g	0.9-1.5 ng/g	85-110	[63]
Cabbage, grape, spinach, and orange spiked	Multi-walled carbon nanotubes	0.001-0.02 mg/kg	0.003-0.05 mg/kg	71-110	[64]

Cabbage, spinach, orange juice, and apple juice	Magnetic MOFs based on Fe_3O_4-MWCNT	0.52-1.83 µg/L	-	62.8-94.2	[65]
Vegetable and fruit juices	Magnetic carbon nano-onions	0.001-0.005 ng/ml	0.003-0.019 ng/ml	82-99	[66]
Rice, cucumber, and tomato	Sorbent formed by magnetic Fe_3O_4 nanoparticle, NH_2-MIL-53(Al), and Chitosan	0.04-0.07 µg/L	0.13-0.23 µg/L	91.25-104.35	[67]

4.3. Solid-phase micro extraction (SPME)

This technique was invented by Arthur and Pawliszyn in 1990 [68]. Since then, various researchers have developed SPE techniques for the quantification of pesticides from foods samples utilizing various solid matrix **(Table 4).** It is a simple sample preparation process that has many advantages, including being free of solvents, being quick, portable, and convenient to use. The separation of analytes between phases bound on SPME fibre and the matrix is the basis of solid-phase microextraction. Zhang et al. (2017) used this technique in combination with "gas chromatography" and a "micro electron capturing detector system" to identify different pyrethroids, including a novel ZIF-90 [69]. The linearity ranged from 0.3 to 50 ng/g, with LODs ranging from 0.1-0.5 ng/g. Using a headspace solid-phase microextraction approach, Kin and Huat (2010) employed a "gas chromatography-electron capture detector (GC-ECD)" technique to analyse pesticide residues in fruit samples like strawberries and vegetables like cucumbers [70]. Saraji et al. (2016) used another SPME technique to analyse organophosphate pesticides (diazinon, fenthion, and chlorpyrifos) [71]. The SPME fibre was employed in combination with a gas chromatography corona discharge ion mobility spectrometer covered with carbon nano tubes having pores. The selected pesticides' LODs were attained in the 0.005-0.020 g/L range. This study showed better extraction performance in comparison with conventional solid phase microextraction fibres such as "polyacrylate" and "polydimethylsiloxane". The method's major limitations include the difficulty of specimen carryover, the delicacy of the fibre, and the extraction process's short lifespan. Also, the extraction process is less effective when there isn't enough plating on the PDMS fibres or contact between the substances in the fibres and the substances in the sample matrix.

Table 4 Application of solid-phase microextraction methods for the detection of pesticides from food samples

Sample	**Matrix**	**Limits of Detection**	**Limits of Quantification**	**Pesticide recovery (%)**	**References**
Cabbage, kale, and mustard	85 µm polyacrylate	0.01-0.14 µg/L	0.03-0.42 µg/L	74.9-114.5	[72]

Strawberry and cucumber	100 μm polydimethyl-siloxane	0.01-1.0 μg/L	0.05-5.0 μg/L	82.7-93.3	[70]
Tomato and lettuce	Magnetic graphene@Fe_3O_4@SiO_2@TiO_2 nanocomposites	0.06-1.49 ng/ml	0.2-4.96 ng/ml	93.89-106.67	[73]
Cucumber, green pepper, Chinese cabbage, lettuce, and eggplant	Molecularly imprinted polymer	0.017-0.77 μg/kg	-	82.1-113.5	[74]
Apple, peach, Chinese cabbage and cucumber	Boron nitride modified multiwalled carbon nanotube	0.01-0.20 ng/g	0.03-0.67 ng/g	83.7-124	[75]
Apple, pear, melon, peach, plum, cucumber, oilseed rape, round lettuce, summer squash, and celery cabbage	1,3,5-triformyl-phloroglucinol and 2-nitro-1,4-phenelyne-diamine	0.04-0.25 μg/kg	0.13-0.83 μg/kg	81.5-111	[76]
Apple juice and tomato	Pencil lead fiber coated with a layer-by-layer graphenized graphite	0.003-0.03 μg/L	0.01-0.10 μg/L	80.7-116.5	[77]
Peach, cucumber and cabbage	Metal-organic framework ZIF-90	0.1-0.5 ng/g	0.3-1.7 ng/g	86.0-103.5	[69]
Apple	100 μm polydimethyl-siloxane	0.01-0.2 μg/kg	0.05-1.0 μg/kg	80.20-105.0	[78]
apple, peach, pear, nectarine, plum, Chinese cabbage, baby cabbage, lettuce, pakchoi, and oilseed rape	Nitrogen-doped porous carbon	0.23-7.5 ng/g	1.13-22.5 ng/g	82.6-118	[79]
Pear, grape, and eggplant	Nanotubes @silicon dioxide nanohybrids	0.005-0.020 μg/L	0.010-0.050 μg/L	79	[71]

Apple and lettuce	carbon nanotubes-doped poly(3,4-ethylene-dio-xythiophene)	15.2-27.2 ng/L	-	87.5-106.5	[80]

4.4. Matrix solid-phase dispersion (MSPD)

Barker and co-workers (1989) was the pioneer in employing this approach for extracting solid and semi-solid materials. Matrix solid-phase dispersion combines removal and cleaning into a single phase, making the technique simple and quick. On the other hand, the optimization of numerous factors, including sample amount, surfactant material, and elusion mixture, is required for this procedure **(Table 5)**. Guan et al. (2011) found nine OPPs from eight different fruit and vegetable samples ("methamidophos, monocrotophos, mevinphos, methidathion, parathion-methyl, malathion, parathion-ethyl, diazinon, ethion") [81]. The proposed method combines matrix solid-phase dispersion with "rapid resolution liquid chromatography-tandem mass spectrometry (RRLC-MS/MS)" for pesticide detection. The technique's recovery range varied from 71.2 to 102.8 %, with RSD ranging from 2.0 to 11.8 %. LODs were discovered to be 0.2 g/kg. Gilbert-López et al. (2010) also compared the modified quick, easy, cheap, effective, rugged, and safe and matrix solid-phase dispersion methods for 105 pesticides [82]. Solid phase microextraction was extracted by liquid balkanization with acetonitrile diluted with petroleum ether, matrix solid-phase dispersion with aminopropyl as an adsorbent, and a florist filter for final clean-up. The technique was quick, easy, cheap, effective, rugged, and safe and included liquid-liquid separation with acetonitrile, dispersive solid-phase extraction, and clean-up using graphitized carbon black, primary secondary amine, and C18 sorbent, with ultimate identification by fast "liquid chromatography-electrospray time-of-flight mass spectrometry (LC-TOF/MS). The revised quick, easy, cheap, effective, rugged, and safe technology exhibited a greater recovery than matrix solid-phase dispersion, according to the data. The LODs were in the range of 0.2-10 g/kg, with a recovery rate of 70-130 %.

Table 5 Extraction and detection of pesticides employing MSPD techniques from various vegetables and fruits foods

Food Sample	**Matrix**	**Limits of Detection**	**Limits of Quantification**	**Pesticide recovery (%)**	**References**
Apple, grape, banana, tomato , strawberry, rape, cabbage, and spinach	Multi-Walled Carbon Nanotubes	0.06-0.15 µg/kg	0.2-0.5 µg/kg	71.2-102.8	[81]
Cabbage, leek, and radicchio	Magnetic graphene oxide	1.1-3.2 µg/kg	0.4-4.0 µg/kg	89-106	[83]
Vegetables	Cucurbit[7]uril	0.13-0.30 µg/kg	0.43-0.99 µg/kg	77.4-110.8	[84]

Apple, pear, and orange	MIP prepared using 4-(dimethoxyphosphorothioylamino)butanoic acid	0.3-1.6 µg/kg	-	81-105	[85]
Lettuce, tomato, carrot, broccoli, cucumber, long bean, celery, spinach, cabbage, and mushroom	UiO-66	0.4-2.0 ng/g	1.4-6.5 ng/g	60.9-117.5	[86]
Tomato, spinacia and cucumber	Graphitized carbon black and primary secondary amine	13.0 µg/kg	43.0 µg/kg	71.9-96.5	[87]
Apple, peach, pear, and plum	Steel Balls-in-tube matrix	3 µg/kg	10 µg/kg	72-113	[88]
Potato, garlic, cucumber, chinese broccoli, lettuce, green bean, fennel, mushroom, and kelp	Neutral alumina-silica gel	0.002 mg/L	0.007 mg/L	86.5-104.8	[89]
Onion	C18 from SPE cartridges	0.003-0.03 mg/kg	0.01-0.1 mg/kg	78.3 to 120.4	[90]
Green vegetables and rice	Unactival C18-bonded silic	0.01-0.015 mg/kg	0.04-0.05 mg/kg	78.1-93.8	[91]
Orange, grape, pomegranate, peach juices, cucumber, and onion juices	Carbonized cellulose nanoparticles	0.31-0.53 µg/L	1.0-1.5 µg/L	92-102	[92]

5. Detection method using instruments

Due to matrix interference, it is challenging to establish a technique for pesticides in actual samples. In recent years, GC and LC have become the most widely used methods for detecting and quantifying insecticides in vegetable foods due to their reactivity, separation, and identification capabilities. Other methods, like ELISA and capillary electrophoresis, have also been used to find pesticide residues in real samples.

5.1. Gas chromatography (GC)

The majority of research claims to have used GC in conjunction with different detectors to identify pesticides. Because of their sensitivity, "electron capture detectors (ECD)", "flame photometric detectors (FPD)" [93], "nitrogen phosphorus detectors (NPD)", and "mass selective detectors (MSD)" are used. To boost technique sensitivity, mass detection methods utilising detectors such as ion trap [94], quadrupole [95], triple quadrupole [96], and time of flight mass analyzer [96] are utilized [97]. Selective ion monitoring (SIM) [98] or "multiple reaction monitoring (MRM)" [99] is also used to reduce matrix interference by concentrating the analyte mass to charge ratio (m/z) to reach a lower threshold of identification and quantitation with less interruption. Husková et al. (2010) suggested combining fast GC and MS to analyze residue levels of pesticides [100]. Using He or N as the gas phase, 90 % of the chromatographic separation is done with fused silica 30 mm×0.2 mm, i.e., 0.25m. In the last ten years, however, the use of GC methods has gone down. This is because more pesticides are being used that are less stable and more toxic. These pesticides are bad for gas chromatography detection methods because they move around and aren't stable at high temperatures.

5.2. Liquid chromatography (LC)

A variety of techniques based on liquid chromatography for examining pesticide residues have been developed, the majority of which combine ultraviolet detectors, photodiode array detectors, diode array detectors (DAD), and mass (MS) detectors. Octadecyl (C18) is often utilised stationary phase for chromatographic multi-residue analysis, and gradient mode has been employed to minimise runtime. Wang et al. (2012) used HPLC with DAD and an Agilent TC-C18 column for separation to quantify seven neonicotinoid pesticides using a multi-residue method [40]. Similarly, Abd Al-Rahman et al. (2012) used HPLC-DAD analysis to investigate the acaricide fenpyroximate's degradation rate in apple, citrus, and grape [101]. Aside from the techniques listed above, Wang et al. (2014) described a method for determining trichlorfon and monocrotophos by HPLC using molecular imprinted solid-phase extraction [102]. Structure information for determining residual pesticide concentrations in fruits and vegetables is difficult to come by, despite the use of liquid chromatography in combination with UV, PDA, and DAD technologies. As a result, employing tandem mass spectrometry, the mass detection approach has been employed to bypass these structural intrusions as well as to offer structural data from molecular masses and disintegration patterns. Different reverse phase columns (C-8, C-12, and C-18), organic phases (acetonitrile and methanol), and buffers have been utilised in a range of research using liquid chromatography with mass detection [5,103]. Ionisation sources such as electro spray ionisation (ESI) are widely used in MS detection because of their capacity to ionise both polar and non-polar substances. For qualitative and quantitative examination, mass analyzers like the "triple quadrupole (QqQ)" [104] and the Q-Trap [105] were utilized. Bakirci and Hişil (2012) developed an approach that used a single quadrupole to evaluate 128 pesticide residues [93]. Another study described a technique for assessing pesticides in fruits and vegetables that used a high-resolution camera, a liquid chromatography-tandem mass spectrometry method based on triple quadrupole-mass spectrometry and electro spray ionization [81]. Furthermore, Tian et al. (2016) presented a technique for real-time assessment of penflufen and one metabolite in vegetables and grains using a revised quick, easy, cheap, effective, rugged, and safe methodology with liquid chromatography-mass spectrometry [106]. An innovative technique for analyzing pesticides

has been published in recent years, employing ultra-performance liquid chromatography due to its chromatographic reactivity and accuracy, in addition to the usage of liquid chromatography-mass spectrometry and tandem mass spectrometry. Sivaperumal et al. (2015) suggested a tool for evaluating chemical residues using electrospray ionization and time of flight detection [107]. For detecting pesticides in fruits and vegetables, Liu et al. (2010) invented triple quadrupole-mass spectrometry using electrospray ionization methodology [53]. Grimalt et al. (2010) proposed a system for quantifying and validating pesticide residue tests adopting ultra performance liquid chromatography in combination with triple quadrupole and hybrid quadrupole time of flight mass spectrometry [41]. In collaboration with Mastovska et al. (2009), they developed a method for the analysis of multiple chemicals in wheat grain using the quick, easy, cheap, effective, rugged, and safe methodology in collaboration with automated direct sample introduction in ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) [108]. Rong et al. (2018) used UPLC-MS/MS to find a way to find pesticides and their byproducts in uncooked foods and fruits and vegetables [104].

6. Optical Screening Methods

6.1. Biochemical Assays

Historically, the use of antibodies or enzymes as detection elements in biochemical experiments has been done in a microplate design, which allows for high output, reactivity, and convenience of use. An excellent example of such bioassays is the enzyme-linked immunosorbent test (ELISA).The foundation of ELISA is the reaction between an enzyme-labeled analyte-specific antibody and its antigen. When a substrate is added to an antibody that has been labeled with an enzyme, a quantifiable colour change occurs. Wu et al. (2019) published a current review that is suggested for a better understanding of the ELISA mechanism, numerous kinds, and recent improvements [109]. Insecticide residues in food matrices have been detected using ELISAs, such as OPs [110], CMs [111], neonicotinoids [112], and fungicides [112,113]. Choline esterases (AChE) and butyryl cholinesterase, (BChE) have been used as recognition elements in cholinesterase microplate assays to screen for pesticides [114]. Because in the same way that competitive ELISAs may correlate the presence of pesticides to a color reduction. Various substrates, such as halides for AChE and BChE have been utilized to produce various coloured products. Cholinesterase microplate assays obtained fewer LODs at the g kg1 level and lower usage of specimens and reagents (usually less than 100 L) depending on the matrix [114–116]. On the other hand, biochemical tests are still useful in labs because they require specific tools and employees who have had a lot of training.

6.2. Biosensor

Biosensors are characterised by instruments that turn a biochemical reaction into a signal that can be measured and processed. In addition to the previously mentioned enticing properties of biological assays tests, biosensors may be downsized and computerized, demonstrating their ability for on-site monitoring. We can differentiate three basic kinds of biosensors based on the biorecognition element, including immune sensors [18], cholinesterase [19], and lipase sensors [115] (enzymatic recognition), and aptasensors [115,117,118]. Aptamers are emerging as a promising alternative to antibodies for addressing concerns such as the difficulty of inducing an

immunological response in tiny molecules or their high temperature stability, which is a concern with biomolecules [119]. Organic solvents, specific pH values, as well as hydrostatic and osmotic pressure, may all harm biomolecules. Better stability can be achieved by binding biomolecules on interfaces just as it can be done with biosensors [120]. The fixation of AChE on cellulose strips, for example, leads to two months of continuous enzymatic activity [121]. Some of the less common recognition parts are molecularly imprinted polymers (MIPs, which are man-made chemicals) and DNA probes.

7. Conclusion and future perspective

An incredible enhancement has been found in the analysis of pesticide remains in vegetables and fruits as a result of the growing value of food safety. Several approaches, including pre-treatment, separation, and screening, are being established. These techniques are time-saving and also decrease the specimen size during the pesticide assessment. Traditional collection and identification methods, as well as mass quantification, are still available as alternatives to extraction and detection procedures. Nonetheless, these traditional techniques take a long time and are expensive. In recent times, various types of advanced methods, for instance, biosensors, molecularly imprinted polymers, and nanotechnology-related techniques, are the best options for pesticide detection in food and vegetables. Nevertheless, in the coming time, various limitations need to be resolved for the development of lucrative and environment-friendly techniques that can identify a higher proportion of pesticide remains in a shorter period.

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Conflict of interest

The authors declare that there is not any conflict of interest with the content of this manuscript.

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