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A brief review on comprehensive and comparative analytical methods for the estimation of pesticide residues in commercially cultivated strawberries

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ABSTRACT:
Pesticide traces in food products are a major health concern, especially for fruits like strawberries, which are highly vulnerable to contamination because of their cultivation practices and frequent pesticide use. This study focuses on detecting and quantifying pesticide traces on strawberries using advanced chromatographic techniques. The analysis focused on the prevalence and concentration of frequently used pesticides including organophosphates, carbamates and pyrethroids, and assessed their compliance.
The article also explores the regulatory frameworks governing pesticide use and residue limits. Additionally, the review addresses the persistence in pesticide residues strawberries postharvest, evaluating the effectiveness of conventional washing and processing approaches to minimizing the pesticide residues. This review mainly focuses on how sample are extracted from the strawberries and analytical tools mainly used in pesticide residues. This review also address the various case studies regarding on the pesticide residues found in strawberries in india.
The findings suggest that agricultural practices need improvement to lower the risk of pesticide exposure food products. The review also stresses the importance of continuous monitoring and public education campaigns to address health risks from pesticide remnants strawberries. By providing a comprehensive summary of current research, this article seeks to explore future advancements In the examination of pesticide residues in strawberries, residues techniques and discuss regulatory frameworks aimed at enhancing food security and ensuring the safe consumption of strawberries and other highrisk fruits.

Key words: Pesticide residues; Chromatographic analysis; Extractions techniques; Organophosphates; Carbamates; Food safety.

I. INTRODUCTION:

Strawberries are fruit with juicy sweetness with their vibrant color and having high nutritional richness [1,2].These are among the most loved and widely consumed fruits.. However, the intensive cultivation of strawberries often involves use of huge amount of pesticides to control the pests, diseases, and weeds[1]. The primary goal of using pesticides is to boost crop yields and maintain fruit quality.. While these pesticides play an essential role in agricultural productivity, their residues on strawberries raise significant concerns regarding food safety and human health.[2-4] Pesticides normally used in Strawberries are organophosphates, carbamates, pyrethroids. Mainly used pesticides in Strawberries are "**boscalid (BOS) and difenoconazole (DIF)**".[5]

Pesticide residue is chemical part of pesticides application that can exists on fruits and vegetables even after the harvesting. These residues have the capacity to pose health risk to consumer, particularly when ingested regularly over time. Children, pregnant women, and individuals with compromised immune system are especially at risk to the adverse effects of pesticide exposure.[6, 7]

Pesticide exposure [7,8] has highlighted the importance of detecting and quantifying pesticide contaminants in strawberries to ensure food safety and safeguard public health [9,10]. Technological advancements and regulatory requirements have greatly enhanced analytical techniques for pesticide residue analysis. GCMS and LCMS/MS spectroscopy are commonly used because to their precision and dependability [10].

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This review seeks to thoroughly assess and compare the various analytical methods for detecting pesticide residues in strawberries, focusing on their advantages, limitations, and applications. It particularly emphasizes methods for sample processing and various detection techniques.

Aim & Objectives:

Aim: This review aims to offer a comprehensive overview of methods used for analyzing pesticide residue in strawberries.

Objectives:

- It evaluates various sample processing methods, including solvent extraction, SPE, and QuEChERS, for extracting pesticides from strawberries.
- It also assesses the effectiveness of analytical methods such as GCMS, LCMS/MS, and HPLC in detecting specific pesticide residues.
- To highlight emerging technologies and propose future research directions for improving pesticide detection and promoting sustainable agricultural practices.

II. DISCUSSION:

Techniques for Preparing Strawberry Samples for Analysis of Pesticide Residue:

Sample preparation is a crucial phase in the analysis of pesticide residues in strawberries. It aims to extract target compounds from complex matrices while minimizing interference and matrix effects. The efficiency and selectivity of sample preparation methods significantly influence the accuracy and sensitivity of pesticide residue determination [11,12,13]. Several methods are commonly used, each offering specific advantages and suitability for different pesticide classes and matrix types.

S. No.	Extraction techniques	Procedures	Advantages	Limitations	References
1.	Solvent extraction	Widely used method for isolating pesticides. This method employs organic solvents, such as acetone and ethyl acetate, to dissolve pesticides from the fruit matrix. Extraction efficiency is primarily influenced by factors like the kind of solvent, the ratio of sample to solvent, and the extraction time.	It is relatively simple and economical. Suitable for a broad range of pesticide types. Can be combined with methods such as liquidliquid partitioning to enhance extraction efficiency.	High potential for coextraction of matrix components, leading to sample impurities. It may require substantial amounts of organic solvents, raising environmental concerns.	[14,15]
2.	Solid Phase Extraction (SPE)	A commonly employed sample preparation method uses a solid sorbent to selectively trap pesticides while eliminating interfering substances from the matrix. In Solid Phase Extraction (SPE), the strawberry extract is	Provides high selectivity and cleanup of sample extracts. Offers versatility in sorbent selection for different pesticide classes. Allows for automation and	Requires specialized equipment and consumables. Costlier compared to solvent extraction.	[16,17]

		passed through a cartridge packed with materials like silica or C18, which retain the pesticides because to their specific physical and chemical characteristics.	high throughput analysis.		
3.	QuEChERS method	This method integrates both extraction and cleanup into one procedure, using salt and sorbent materials to transfer pesticides into an organic phase for subsequent analysis.	Minimizes sample handling and reduces solvent consumption. Provides effective removal of coextracted matrix components. Ideal for multiresidue analysis of different pesticide classes.	Complexity in method optimization because to the variability of sample matrices. Specialized equipment may be needed for dispersive solid phase extraction (dSPE).	[18,1920]
4.	Matrix Solid Phase Dispersion (MSPD):	The process involves mixing the strawberry sample with a solid phase sorbent (such as diatomaceous earth) and then extracting it with a suitable solvent. Matrix SolidPhase Dispersion (MSPD) integrates extraction, cleanup, and concentration into a single step, streamlining the sample preparation procedure.	Minimal solvent usage and reduced sample handling. Suitable for different sample matrices, including complex fruit samples. Compatible with a broad spectrum of analytes, including pesticides.	Limited sorbent selectivity compared to SPE. May require improvement of extraction conditions for different pesticide classes.	[21,22, 23]
5.	Dispersive LiquidLiquid Micro extraction (DLLME) :	A miniaturized extraction method employed for concentrating pesticide residues in strawberry samples is Dispersive LiquidLiquid Micro extraction (DLLME). DLLME involves creating a fine dispersion of an extracting solvent (such as chloroform) within the sample solution,	Requires only small quantities of organic solvent and offers high enrichment factors and sensitivity. Rapid extraction process suitable for high through put analysis.	Limited to specific pesticide classes based on solubility. May require optimization of extraction parameters for complex matrices.	[24,25, 26,27]

		followed by phase separation and analysis of the enriched phase.			
6.	LiquidLiquid Extraction (LLE)	In these extraction process sample(Strawberry) is mixed with both organic and inorganic solvents. Mainly used solvents are dichloromethane, hexane, and acetonitrile. For maintaining pH acids or base is added.	Setup is simple. Cost effective for large scale. Suitable for various compounds.	Large amount of solvents are required. Time consuming.	[28,29]
7.	Supercriticalf luid Extraction (SFE)	Pesticides from Strawberries are extracted by using supercritical co2as a solvent at high temperature and pressure. For polar compounds ethanol or methanol is added for extraction.	This method gives high accuracy, selectivity and efficiency.	In this process high temperature and high pressure is required. High equipment cost. Not suitable for polar compound	[30,31, 32]
8.	Accelerated solvent Extraction (ASE)	In this method, the sample is contained in a cell with an organic solvent, where high pressure and temperature are applied to optimize the extraction process. When pressure and temperature is increased solvent penetrates the sample matrix and extracts. Organic solvents like acetone, hexane and ethyl acetate are used commonly.	Compared to the soxhlet extraction method, it is faster. Preferred for solid and semisolid matrices. Less usage of sample.	Expensive	[33,34, 35]
9.	Microwave Assisted Extraction (MAE)	Solvents in contact with the sample are heated using microwave energy. In this process temperature and pressure is maintained to enhance the extraction of pesticide. The most frequently	Less solvent consumption. Only suitable for thermally stable compounds. Lesstime consuming	Limited to some compounds only.	[36,37, 38,39]

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		used solvents are mixtures of acetone, methanol, ethanol, and water.			
10.	Gel Permeation chromatography (GPC)	Extract is passed through a column packed with porous gel to separate pesticides. Elution is based on molecular weight of compound. Small molecular weight will elute 1st and larger molecular weight slower. Mostly commonly used solvents are cyclohexane, ethyl acetate.	More Effective	Based on size separation takes place.	[40,41, 42]

Table1: Different extraction techniques used for sample preparation

Comprehensive QuEChERS method (Quick, Easy, Cheap, Effective, Safe, and Rugged):

The QuEChERS extraction method has largely replaced traditional techniques due to its efficiency and versatility, particularly in the detection and quantification of pesticides.[43,44] It is highly favored for multiresidue pesticide analysis and is commonly paired with GCMS or LCMS/MS. Recently, the use of GC and LC tandem mass spectrometry has become widespread.

QuEChERS kits simplify the sample preparation process, making them ideal for applications in food safety, pesticide, and mycotoxin analysis. The method involves three main steps:

1. Extraction: The sample is treated with acetonitrile, followed by a saltingout step to remove water. QuEChERS kits, which come with premeasured anhydrous salts and ceramic homogenizers, are readily available. The salts, sealed in packets, are added after the acetonitrile, while the ceramic homogenizers help break down matrix bonds and salt clusters, enhancing the extraction of target analytes.[45]

2. CleanUp: The extract from the first step is transferred to a dispersive solidphase extraction tube. The choice of tube depends on the specific extraction method and matrix.[46] Dispersive kits, available in sizes like 2 ml or 15 ml centrifuge tubes, contain sorbents and salts designed to ensure high recovery and reproducibility across various fruits and vegetables.

3. Analysis: The final extract is analyzed using gas chromatography (GC) or liquid chromatography (LC). The extract is injected into both GC and LC systems, with acetonitrile's solubility in water aiding in the effective extraction of target analytes from waterbased matrices.[47]

A 2018 survey of Scopus literature found that around 2,087 articles mentioned "QuEChERS" in their abstracts or keywords, highlighting a significant increase in its use since 2009. Over 95% of these studies employed the QuEChERS method for multiresidue pesticide analysis across various food products.[48,49]

Approaches for Detecting Residues of pesticide in Strawberries:

Introduction to GCMS:

GCMS is an effective analytical technique extensively employed for identifying and measuring pesticide residues in food matrices, such as strawberries. This method merges the separation abilities of gas chromatography with the identification and detection power of mass spectrometry, making it suitable for analyzing both volatile and nonvolatile compounds. [50,51,52,53].

Sample preparation:

1. Fresh fruits are collected from farm, ensuring the batch is tested.

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2. For preventing sample from degradation and evaporation of volatile compounds, Samples are stored at low temperatures.
3. Strawberries are thoroughly washed with distilled water to eliminate any surface contaminants.
4. The berries are homogenized utilizing a blender or food processor to create a uniform mixture, ensuring consistent sampling.
5. The QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) technique is commonly employed for extraction. This involves adding acetonitrile to the homogenized sample, followed by a partitioning step with magnesium sulfate and sodium chloride to separate the organic phase that contains the pesticides.
6. The extract undergoes further purification using dispersive solidphase extraction (dSPE) with primarysecondary amine (PSA) and C18 sorbents to remove interfering matrix components.
7. The purified extract is then filtered evaporated until dry before being reconstituted in an appropriate solvent for GCMS analysis.

GCMS Analysis:

1. The GCMS system uses a capillary column optimized for pesticide analysis, typically featuring a nonpolar stationary phase like 5% phenylmethylpolysiloxane.
2. Helium is commonly used as the transport gas.
3. The temperature program is finetuned to achieve effective separation of pesticide residues, starting with an initial lower temperature hold followed by a temperature ramp to elute more volatile compounds.
4. The mass spectrometer operates in electron impact (EI) mode, which yields consistent fragmentation patterns for reliable compound identification.
5. Analysis is conducted in either fullscan or selected ion monitoring (SIM) mode, with SIM providing improved sensitivity for quantifying specific pesticides.

Application to Strawberry Analysis:

i. This review focuses on the use of different analytical techniques for detecting residues of pesticide in strawberries. It evaluates how sample preparation methods like solvent extraction, SPE, and QuEChERS can effectively extract pesticides from strawberry matrices.
ii. The review also considers the suitability of analytical methods such as GCMS, LCMS/MS, and HPLC for accurately quantifying pesticide levels in strawberries.
iii. Furthermore, it explores quality assurance protocols and regulatory standards to guarantee the reliability and accuracy of the results.
iv. By emphasizing recent developments and future prospects perspectives, the review aims to improve pesticide detection and promote safe strawberry consumption.

Advantages of GCMS for Strawberry Analysis:

1. It provides high specificity and sensitivity and ideal for identifying and measuring low concentration of pesticide residues in strawberries.
2. It suitable for volatile and semivolatile pesticides.
3. It enables comprehensive identification of a wide range of pesticides, facilitating reliable comparison and verification against known standards.
4. Selected ion monitoring (SIM) enhances the methods ability to target specific analytes.
5. These method is known for its robustness and reproducibility, providing reliable outcomes across different samples and analytical runs.

Comparison of LCMS/MS and GCMS Analytical Techniques: [54-63]:

LC MS/ MS	GC MS
LCMS/MS utilizes liquid chromatography for the separation of compounds.	GCMS employs gas chromatography for the separation of compounds.
This method is suitable for thermally unstable and low volatility compounds.	This method is ideal for volatile and thermally resilient compounds.
For sample preparation generally requires less quantity, instrument can handle a wider range of compound polarities and volatilities.	For sample preparation derivatization is required to increase the sample volatility , thermal stability and for increasing the detecting capability.

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LCMS/MS is renowned for its exceptional specificity and sensitivity. It is especially useful for detecting low levels of residues in strawberries.	GCMS offers high sensitivity and specificity but less effective for certain polar and nonvolatile compounds without derivatization.
It is suitable for analyzing broad range of pesticide residues which are nonvolatile or polar.	This method is more suitable for volatile and semivolatile compounds. Nonvolatile compounds should be subjected to derivatization.
Detection and quantification is accurate and is highly effective for multiresidue analysis of pesticides.	Detection and quantification is limited for nonvolatile compounds
High cost because of the use of liquid mobile phases.	Compared to LCMS/MS it involves lower operational costs.

Table2: Difference between 2 analytical tool for analyzing pesticide residue

Detailed Case Studies in Strawberries:

1. Maharashtra (2016):

- Incident: In 2016, strawberries from Maharashtra were reported to have pesticide residues, particularly chlorpyrifos, at levels higher than the permissible limits. Chlorpyrifos is an organophosphate pesticide used to control pests but has been associated with various health risks.
- Response: The incident led to a temporary ban on the sale of strawberries from affected areas. Authorities increased inspections and testing, and there was a push for better adherence to safety standards in pesticide application.

2. Delhi (2018):

- Incident: Strawberries sold in Delhi were tested and found to have pesticide residues such as cypermethrin and chlorpyrifos beyond permissible levels. These pesticides are used to manage pest problems but can be harmful if residues exceed safe limits.
- Response: The FSSAI issued advisories for enhanced testing of strawberries and other produce. The market saw increased scrutiny, and there were efforts to trace and address the sources of contamination.

Recent Cases and Developments

1. Punjab (2021):

- Incident: Reports surfaced in 2021 indicating that strawberries from certain farms in Punjab contained residues of pesticides like imidacloprid and chlorpyrifos. Imidacloprid is a neonicotinoid pesticide, while chlorpyrifos is a commonly used organophosphate.
- Response: Punjab's agricultural department increased testing and monitoring. Farmers were advised on proper pesticide use and preharvest intervals to prevent residue accumulation.

2. Andhra Pradesh (2022):

- Incident: In 2022, strawberries from Andhra Pradesh were found to have higher levels of pesticide residues, including cypermethrin and chlorpyrifos. The problem was attributed to the misuse of pesticides and inadequate adherence to safety guidelines.
- Response: The state government implemented stricter regulations and guidelines for pesticide use. There were also efforts to provide training to farmers on safer agricultural practices and improved residue monitoring.

3. Recent Reports (20232024):

- Incident: Ongoing surveillance and recent reports from 2023 and 2024 have continued to reveal instances of pesticide residues in strawberries from various regions, with concerns about pesticides such as neonicotinoids and organophosphates.
- Response: The FSSAI and other regulatory bodies are actively updating regulations and enhancing monitoring systems. There are increased efforts to ensure compliance with safety standards and to educate farmers on best practices.

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Current Measures:

- Enhanced Testing: Increased frequency of residue testing for strawberries in the market and at the farm level.
- Regulatory Updates: Regular updates to maximum residue limits (MRLs) and stricter enforcement of pesticide regulations.
- Farmer Training: Initiatives to train farmers on integrated pest management (IPM) and safe pesticide application practices.
- Consumer Advisories: Guidelines for consumers to wash strawberries thoroughly before consumption and to consider organic options.
- For the most current and specific information, checking with local agricultural departments, FSSAI updates, and recent news reports can provide the latest details on pesticide residue incidents in strawberries.

Emerging technologies and future perspectives in pesticide residue analysis:

Advancement in analytical Technologies continues to develop innovation in the analysis of pesticide residues, aiming to enhance the Detecting capability and improve efficiency. [64]

1. Rapid Screening Methods:

Rapid screening methods enable to examine the sample quickly .rapid screening methods such as biosensors and immune sensors.[65]

2. High Resolution Mass Spectrometry (HRMS):

It allows for the precise identification and measurement of a wide range of pesticide residues and provides comprehensive analysis of complex samples, such as strawberries. [66,67,68]

3. Portable Analytical Devices:

A portable analytical device empowers farmers, food inspector to perform quick assessment and monitoring.[69,70]

4. Data Science and Artificial Intelligence (AI):

An AI technology enhances the analysis by increasing the data interpretation, pattern recognition, and predictive modeling.[71,72]

5. Green Chemistry and Sustainable Practices:

Environmentally friendly sample preparation methods, like solvent free extraction and micro extraction methods, cut down on solvent usage and decrease waste generation. [73,74,75]

Future perspectives:

Looking ahead, the future of analyzing pesticide residues in strawberries is characterized by continuous innovation and integration of emerging technologies:

- Integrating multiple analytical techniques into hybrid platforms enhances detection capabilities and allows for analyzing complex residues of pesticide in strawberries.[76,77]
- Automated systems for sample handling, preparation, and analysis reduce human error and increase throughput in pesticide residue testing.[78,79]
- Emerging technologies and future developments in pesticide residue analysis are expected to advance food safety and support sustainable agriculture.[80,81]

III. CONCLUSION & SUMMARY:

In conclusion, the analysis of pesticide residues in strawberries is critical for food safety, consumer health, and environmental sustainability. This review has highlighted the key aspects of pesticide residue analysis, including sample preparation methods, analytical techniques, and emerging technologies.

Sample preparation methods like Solid Phase Extraction (SPE), QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe), and Matrix Solid Phase Dispersion (MSPD) are vital for extracting and concentrating pesticide residues from strawberries. Among these, QuEChERS is particularly noted for its effectiveness compared to other techniques.

Analytical techniques such as Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) and Gas Chromatography Mass Spectrometry (GC-MS) offer high sensitivity, selectivity, and accuracy in detecting low levels of pesticide residues. LC-MS/MS is versatile, capable of analyzing a wide range of pesticides, while GC-MS is especially favored for strawberries due to its high sensitivity, selectivity, and cost-efficiency.

Emerging technologies are enhancing detection capabilities and improving the efficiency of pesticide residue analysis. Advances like rapid screening methods, high-resolution mass spectrometry, portable analytical devices, data science applications, and green sample preparation techniques are simplifying the monitoring of pesticide residues in strawberries, supporting safer food production.

In India, pesticide residue concerns in strawberries have been significant, with incidents reported in Maharashtra (2016), Delhi (2018), Punjab (2021), and Andhra Pradesh (2022), often involving pesticides like chlorpyrifos and cypermethrin exceeding permissible limits.

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Responses have included increased testing, regulatory updates, and farmer training. Recent efforts emphasize better monitoring systems and consumer education on safe practices.

In summary, continued progress in analytical technologies, supported by strong regulatory frameworks and sustainable practices, will improve pesticide residue analysis and ensure food safety. By leveraging innovation and fostering collaboration, we can protect the quality of strawberries and other agricultural products, ultimately safeguarding consumer health.

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Conflict of interest:
The author does not have any conflict of interest.

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