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Advancements and challenges in extraction and determination of pesticide residues in soya products

Agneša Szarka, Barbora Vidová, Nicolette Viktoryová, Svetlana Hrouzková

Slovak University of Technology in Bratislava, Faculty of Chemical and Food Technology, Institute of Analytical Chemistry, Radlinského 9, 812 37 Bratislava, Slovak republic
svetlana.hrouzkova@stuba.sk

Abstract: Soya beans, a vital source of protein, oil, and dietary fibre, are cultivated extensively, with a growing demand since 1950. However, the extensive use of pesticides in soya bean cultivation poses potential risks to human health and the environment, especially given the lack of strict regulations for maximum residue levels in soya-based products within the European Union. This study provides a comprehensive overview of isolation and determination methods of pesticide residues from soya products, emphasising the challenges posed by the high-fat content of soya beans. Various chromatographic techniques coupled with mass spectrometry are discussed for their efficacy in pesticide residues analysis. The manuscript also reviews sample preparation techniques, with a focus on the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method, and its modifications to enhance extraction and purification efficiency. Additionally, the study explores the application of liquid-liquid extraction and solid-phase extraction in the analysis of soya oil and beverages and innovative methods, such as dispersive liquid-liquid microextraction and matrix solid-phase dispersion, for comprehensive pesticide residue analysis. This review highlights the importance of multiresidue analytical methods in ensuring the safety of soya products thereby supporting regulatory compliance and public health protection.

Keywords: soya products, pesticides, sample preparation, real sample analysis

Introduction

The vegetarian diet is popular among the population. Vegetarians supplement their diet with legumes (e.g., soy, lentils, peas), nuts (e.g., cashews, macadamia, almonds, pecans, walnuts) and seeds (e.g., chia, flax seeds) or pseudocereals (quinoa, buckwheat). The range of products includes vegan foods such as dairy alternatives (e.g., oat, almond, soy drinks) and cheeses or meat substitutes (e.g., soy-based). A significant part of vegetarian diet is soya beans and various soya products. Soya beans (*Glycine max* (L.) Merr.) have been grown in the Far East since early times and have become of supreme importance as a source of oil and protein throughout the world during the 20th century. Soya bean is considered one of the most widely consumed legume crops in the world. Usually, soya seeds consist of about 36.5 % protein, 19.9 % lipids, 30 % carbohydrates, and 9.3 % dietary fibre. According to information from the World Wildlife Organization, the demand for soya beans has increased up to 15 times since 1950. However, increased use of pesticides in soya bean cultivation is also related to this increased demand. The use of pesticides presents a danger of residues in the final products, posing a risk to the environment and human health. To guarantee the safety of food and feed and to regulate international trade, the European Union (EU) has set maximum residue levels (MRLs) to minimise the presence of pesticide residues in various foods. Agricultural practice in soya bean production requires large amounts of pesticides and there is no current legislation establishing MRLs for pesticide residues in soya-based products, which makes it difficult to control the indiscriminate the use of these compounds and to preserve health of the consumers.

Determination of pesticides in high-fat samples such as soya beans is very complicated, therefore, prior to the actual sample analysis, it is very important to treat the sample with a suitable extraction technique. Chromatographic techniques in conjunction with mass spectrometry (MS) are commonly used to analyse extracts as they provide satisfactory selectivity and sensitivity.

The aim of the study is to provide an overview of methods for the detection and determination of pesticides in soya bean products and of sample preparation techniques used for sample analysis to detect and determine pesticides at low concentration levels. The paper critically evaluates the up-to-date achievements and the authors set challenges for the near future in this area.

Soya and soya products

Soya is an annual legume belonging to the *Fabacean* family with edible seeds. Soya beans are among

the six most useful agricultural crops in the world because they are used as food for humans and animals, but also as a raw material for biodiesel production (Augustyn, 2019).

Soya beans were first cultivated in America in 1804 and remained a rare garden plant for nearly 100 years. They can be grown in almost all types of soil, but produce best in warm, fertile, well-drained sandy loam away from the danger of frost. Like other legumes, soya beans enrich the soil with nitrogen (Rigby, 2001).

Soya products and soya bean oil became very scarce food during World War II when the demand for edible oil increased. Nowadays, soya bean oil is also used in industry, mainly in the production of paints, varnishes, and other drying oil products (Shurtleff, 2009).

Soya bean oil and soya bean meal are a source of fuels and bioproducts. Consumer interests and political interests are focused on achieving high content of biological materials in consumer goods to meet the growing expectations regarding sustainability and the use of renewable resources. Along with these consumer and legislative trends, stricter environmental standards, increased ability to provide improved performance characteristics, and more cost-effective chemical conversion processes lead to increased use of soya products as raw materials and materials for industrial products. These factors provide motivation to develop new materials from soya beans, which may create new markets (Schmitz et al., 2008).

Generally, soya bean proteins represent 30—60 % on dry weight basis; e. g., soy milk contains around 3—4 % of protein (Olías et al., 2023) while storage proteins are the most prevalent. β-Conglycinin (7S) and glycinin (11S) globulins are two storage proteins that make up 80 % of the total protein content of soya. In addition, there are other less abundant storage proteins such as 2S, 9S, and 15S globulins. In addition to these proteins, soya also contains enzymes, protease inhibitors, lectin, and others (García et al., 1997).

Soya beans are abundant in lipids, representing up to approximately 18—24 % of their total dry weight. Storage lipids are deposited mainly in the form of triacylglycerols in oil bodies. A triacylglycerol molecule is composed of three fatty acids esterified to a glycerol backbone. The majority of fatty acids (FAs) found in mature soya beans are unsaturated fatty acids, accounting for approximately 85 % of the total lipid content. Among them, linoleic acid (C18:2n C6) is the most abundant, making up to 50 % of the total fatty acid content (Olías et al., 2023), followed by oleic (18:1), palmitic (16:0), linolenic (18:3), and stearic (18:0) acids (Medic et al., 2014).

Soya beans contain a significant amount of isoflavones, with the main isoflavone compounds, genistein, daidzein, and glycitein, occur in four chemically different forms as aglycone, β-glucoside, acetyl-β-glucoside, and malonyl-β-glucoside. Soya isoflavones have the ability to alleviate symptoms associated with menopause and prevent age-related dysfunctions such as cardiovascular disease and osteoporosis in postmenopausal women, among others, by exhibiting antioxidant and anticarcinogenic effects. On the other hand, there is growing concern about excessive human exposure to isoflavones due to their estrogen-like properties. The presence of isoflavones has also been reported in soya bean leaves, branches, pods, and roots obtained from living plants (Carneiro et al., 2020).

Various diseases and plant pests can have negative impact on the production of soya products. Losses caused by insect damage reduce grain yield and quality and thus also seed quality. Today, grain quality is not an important factor in the sale of soya beans, but this may change in the future. There has been limited research on the effects of disease and insects on these traits, which will change as markets for these traits develop and rapid and accurate assessment of these traits is required. Most of these diseases and insects directly affect soya beans (Rupe, 2008).

Nowadays, new soya bean varieties are developed with the help of hybridisation and genetic modification. Hybridisation enables the isolation of types that provide good yields, are resistant to diseases, and are adaptable in different maturity requirements as well as in different environments. Genetically modified soya beans are engineered, e.g., to be resistant to glyphosate and are among the most widely used genetically modified organisms (GMOs). To ensure soya bean production and prevent pests, a large number of pesticides are used, which can then also be present in form of residues in the final soya bean products, thus endangering consumers (Rigby, 2001).

Presence of pesticide residues in soya and soya products

Pesticide presence in soya and soya products is a significant concern due to the extensive use of chemicals such as glyphosate in soya farming, especially with genetically modified (GM) soya beans (Rigby, 2001). Pesticides can enter soya products through several pathways during cultivation, harvest, and processing. Direct application of pesticides to soya crops, absorption from contaminated soil and water, and air drift are primary sources during cultivation. Post-harvest handling and processing can also introduce pesticide residues through

contaminated equipment and cross-contamination (Páleníková et al., 2015). Environmental factors, such as contaminated soil and water, as well as transport and storage practices, further contribute to the presence of pesticides. Regulatory bodies set MRLs to ensure safety, and most soya products meet these standards. Regular testing is conducted to monitor pesticide residues using methods such as gas chromatography and mass spectrometry.

To minimise contamination, integrated pest management (IPM), organic farming, thorough washing, and proper processing are essential, alongside regular testing and monitoring to ensure that safety standards are met.

Organic soya beans, grown without synthetic pesticides, have lower residues but are not entirely free from contamination. Health risks associated with pesticide residues include endocrine disruption and reproductive issues leading to increased consumer demand for organic and non-GMO soya products.

Sample preparation of soya-based samples

Samples of soya and soya-based products can occur in different forms, with different content of potential interferences (from the point of analysis) in the matrix, therefore analytical methods are here divided into subsections according to the type of matrix. An overview of the analytical methods is summarised in Table 1, where, in addition to samples and types of analytes, extraction techniques, cleaning techniques and instrumental techniques for separation and detection of analytes are listed. The limits of detection and detection of pesticides in real samples are also provided.

Soya beans

Most studies in the field of soya bean product analysis have focused on the analysis of raw soya beans, while up to 40 % of literature reviewed pesticides determination in these products (Table 1). The determination of pesticide residues in soya beans is crucial for ensuring food safety and compliance with regulatory standards. Soya beans, being a major agricultural product, are subject to pesticide application, making it essential to monitor residue levels effectively. Advanced analytical techniques have been developed to detect and quantify these residues with high precision and sensitivity.

For soya bean samples, the most frequently used sample preparation method is the quick, easy, cheap, effective, rugged, and safe (QuEChERS) sample preparation process, which was originally developed by Anastassiades et al. in 2003 (Anastassiades et al., 2003). It has been used for multiresidue pesticide analysis of a wide variety of plant and animal-based matrices. QuEChERS involves a salt-out acetonitrile extraction/partitioning step, followed by a clean-up step using dispersive solid phase extraction (dSPE) with primary secondary amine (PSA) prior to the analysis by gas chromatography-mass spectrometry (GC-MS) or liquid chromatography-tandem mass spectrometry (LC-MS/MS). In the traditional QuEChERS method, acetonitrile is used as the extraction solvent, which is also the preferred option for soya bean samples analysis, however, it was mixed with water in a 1:1 ratio in some cases. Soya bean samples represent a very complex matrix for GC-MS, LC-MS analysis and therefore, the cleaning step (dSPE) is very important. Different types of sorbents have been applied to eliminate interferences from the acetonitrile extract using mostly PSA in combination with other sorbents. The combination of PSA and Florisil was compared with the combination of PSA, Florisil, and C18 (Huertas Perez at al., 2015). The mixture of PSA and C18 sorbents was found to be insufficient for the elimination of matrix effects, where three pesticides, tebuconazole, diazinon, and chlorpyrifos, showed matrix suppression. On the other hand, with the combination of the three sorbents, the recoveries of problematic pesticides ranged between 60 and 75 %, while the recovery of imidacloprid increased up to 90 %. Other compounds were extracted with recoveries ranging from 80 to 90 % (Huertas Pérez et al., 2015).

The QuEChERS procedure without a purification step was used to isolate a mixture of pesticides from soya beans. Acetonitrile with the addition of 1 % acetic acid along with $MgSO_4$ and NaOAc was used for extraction. Satisfactory separation of pesticides (thiabendazole, aminocarb, imazil, methoxuron, carbofuran, imazapyr, and metosulam) was obtained after the addition of 100 mM acetic acid (pH 2.4). Recoveries of the pesticides ranged between 85—120 % with a relative standard deviation (RSD) of less than 6.1 % in all cases. The limits of detection (LODs) were lower than 0.1 μg·kg for all pesticides tested, even several times lower than the established MRLs for pesticides in soya beans (Daniel, 2018).

Herrera López et al. (2019) compared different types of sorbents for the purification of soya bean methanol extract. Several sorbents, EMR-Lipid, CAPTIVA, weak cation exchange sorbents (WCX), hydrophilic-lipophilic balanced sorbent (HLB), and a reversed-phase sorbent operating with strong cation exchange (MCX) were tested in the purification process to evaluate their concentration factors and matrix effects. None of the sorbents alone proved suitable for the isolation of all pesticides, and a combination of the two was used for final pu-

rification providing good separation and improving the shape of the chromatographic peaks. Reliable recoveries in the range of 70—120 % were obtained by combining the sorbents. The extracts were analysed by hydrophilic interaction chromatography in combination with MS. An analysis of real samples showed that this method is reliable and can be used in practice.

A new and environmentally friendly method determining pesticide residues in soya beans has been developed by Petrarca et al. (2024), offering a reliable alternative to conventional extraction techniques. This innovative approach integrates the classical QuEChERS extraction method with a dispersive liquid-liquid microextraction (DLLME) step, utilising a deep eutectic solvent (DES) made of camphor and hexanoic acid in a 1:1 molar ratio. This DES has not been previously used in pesticide analysis of complex matrices such as soya beans through gas chromatography-mass spectrometry (Petrarca et al., 2024).

A method based on the determination of pesticides by gas chromatography (GC) combined with isotope dilution (MS) was used for the detection of diazinon, malathion, chlorpyrifos, captan, alpha-endosphan, pendosulphan, iprodione, and cypermethrin in soya beans. Ethyl acetate was used as the extraction solvent, then the extract was dried with $MgSO_4$ and injected into the chromatographic system. The method was not suitable for the determination of tebuconazole as the presence of both the quantifier and qualifier ion was interfered by impurities, but it was possible to detect all the other pesticides investigated. The recoveries ranged from 80 to115 % (Huertas-Pérez et al., 2019).

Maldaner et al. (2008) used the matrix solid-phase dispersion (MSPD) technique, which involves dispersing a sample over a solid support and eluting the compounds with a small volume of solvent. The major advantage of the method is that extraction and purification can be performed in a single step, reducing the overall analysis time. Silica was added to the soya bean sample, the mixture was mixed and injected into propylene syringes containing the co-adsorbent. The syringes were connected to vacuum and pesticides were eluted with ethyl acetate and methanol. Subsequently, the extract was dried with nitrogen and dissolved in acetonitrile. The final extract was then analysed by high performance liquid chromatography (HPLC) (Maldaner, 2008).

Hong et al. (1993) chose to determine pesticides in soya beans by the GC-MS method. A sample of 25 g was ground and extracted with a 2:1 mixture of solvent, acetone and methanol. After filtration through a Buchner funnel, the filtrate was evaporated on a rotary evaporator and water and saturated sodium chloride solution were added. After cleaning the sample with SX-3 sorbent, the sample was dried under a stream of nitrogen and dissolved in 2 ml of hexane. This method of analysis gave reliable recoveries with reasonable precision, and it appears to be suitable for the determination of a mixture of several pesticides. Each pesticide was identified by retention time and characteristic fragment ions using GC-MS (Hong et al., 1993).

From this chapter it is evident that various modifications of the QuEChERS method have been commonly used to analyse soya beans for pesticide extraction. It has been shown that using a mixture of different sorbents, the best purification of the extracts and removal of fat particles from the matrix can be achieved. Different chromatographic techniques, mainly GC and LC coupled with MS, are used for extract analysis. Table 1 summarises a review of articles on the determination of pesticides in soya beans.

Soya oil

Pesticide residues determination in soya oil is a critical aspect of food safety, ensuring that the oil is free from harmful levels of pesticide residues. The complexity of soya oil due to its high fat content and the potential for various types of pesticides presence requires sophisticated analytical techniques. The goal is to achieve accurate, sensitive, and reliable detection and quantification of pesticides. The extraction of pesticides from soya oil often involves liquid-liquid extraction (LLE) or solid-phase extraction (SPE) (Table 1). These techniques aim to separate pesticides from the oil matrix. Common solvents used include acetonitrile, hexane, and ethyl acetate. Post-extraction, a clean-up step with different sorbents is necessary to remove interfering substances.

Dias et al. (2016) conducted a comparative study on different sorbents, evaluating their efficiency in eliminating fats from soya bean oil samples. Utilising the QuEChERS method to extract 165 pesticides, they compared PSA, Z-Sep, and a specialised sorbent, EMR-Lipid, in the purification step. Subsequent analysis by UHPLC-MS/MS revealed that EMR-Lipid offered significant advantages over other sorbents, including more reliable pesticide recoveries and higher repeatability, with recoveries ranging from 70—120 % and RSD <20 % for most pesticides (Dias et al., 2016).

Further research explored a combination of matrix-assisted solid-phase dispersion (MSPD) with mini-column size exclusion chromatography (M-CS-EC) for pyrethroid pesticides separation from soya bean oil. Using an Extrelut-3 cartridge packed with diatomaceous earth and acetonitrile as the extraction

solvent, this method allowed for efficient fat removal and reduced solvent consumption. Di Muccio et al. (1999) reported recoveries of 80—111 % for nine out of 14 pesticides investigated (Di Muccio et al., 1999).

In conclusion, the determination of pesticides in soya oil is essential for ensuring food safety, given the complexity of soya oil fat content and the variety of pesticides used. Accurate, sensitive, and reliable detection methods are critical, often involving LLE or SPE to separate pesticides from the oil matrix. Advanced techniques such as UHPLC-MS/MS and various clean-up procedures, including the use of specialised sorbents like EMR-Lipid, have shown effectiveness in achieving high recoveries and repeatability. Comparative studies highlight the advantages of different extraction and purification methods, such as SMDP combined with mini-column size exclusion chromatography for specific pesticides such as pyrethroids.

Soya milk and other soya-based beverages

Soya milk is a plant-based beverage which became popular as the alternative to cow's milk and it is gaining popularity among people with lactose intolerance and milk protein intolerance. Soya milk is rich in proteins, vitamins, minerals, and the content of lipids is relatively low compared to the original soya beans as lipid content of soya milk is about 2 %. The increased consumption of soya beverages in Brazil and associated pesticide concerns prompted the use of the UHPLC-MS method for pesticide content determination. The QuEChERS method, using 10 ml of acetonitrile, was optimised for purification with C18 and PSA sorbents. May et al. (2017) observed that C18 provided recoveries of pesticides closer to 100 %, with LOD and LOQ ranging from 3—8 $\mu g \cdot L^{-1}$ and 10—25 $\mu g \cdot L^{-1}$, respectively. The chosen combination of QuEChERS and UHPLC-MS methods was found to be suitable for the determination of larger amounts of pesticides in soya beverages in routine laboratory practice (May et al., 2017).

Campillo et al. (2015) investigated the determination of seven strobilurin fungicides in soya beverages using DLLME. Precipitation of proteins in an acidic medium was necessary to prevent analytical interference. After extraction and clean-up, LC with a diode array detector (DAD) was used for the analysis, although none of the fungicides were detected in real samples (Campillo et al., 2015).

The effect of NaOH addition on the pesticide extraction was studied in the determination of pesticides in soya milk by gas chromatography coupled with a flame ionisation detector (GC-FID). For a suitable extraction solvent, the following properties were emphasised: miscibility with aqueous solution, immiscibility with n-hexane, and high extraction efficiency in the presence of aqueous solution and n-hexane. Therefore, four different solvents, i.e. acetonitrile, acetone, methanol, and isopropyl alcohol, were investigated, demonstrating that only ACN formed a three-phase system in the presence of n-hexane after the addition of the Na_2SO_4 reagent. During the pesticides' extraction, n-hexane was shown to be partially dissolved in acetonitrile and the organic phase was collected at the top of the aqueous solution due to its lower density. Removal of the organic phase with the extracted analytes was problematic, and thus, NaOH was added in different volumes and concentrations. Best recoveries were achieved at the NaOH concentrations of 0.1 $mol \cdot L^{-1}$ and volume of 500 µL (Abbaspour et al., 2019a).

Hernández-Borges et al. (2005) determined herbicides in soya milk using capillary electrophoresis (CE) combined with MS. Following dilution, acidification, and SPE extraction, herbicides were eluted with acetonitrile and analysed, achieving LODs at ppb levels with RSD between 3.8 and 8.1 % (Hernández-Borges et al., 2005).

Rejczak et al. (2016) used HPLC-DAD for sulphonylurea herbicide determination in soya milk. Following acetonitrile extraction and Z-Sep purification, recoveries ranged from 61—108 % with RSD <15 %. Chlorosulphuron was detected in real samples at 14.2 $ng \cdot mL^{-1}$ (Rejczak, 2016).

Gionfriddo et al. (2020) introduced a novel DI-SPME (direct immersion solid-phase microextraction) method for soya milk analysis, a representative matrix for food commodities high in proteins and lipids. The method employed a matrix-compatible SPME fibre (PDMS/DVB/PDMS), which outperformed PDMS/DVB fibres in coating robustness and repeatability for extracting pesticides used on soya plants. Key to the method's development was optimising the SPME fibre cleaning protocol, involving a 10-second pre-desorption rinse in a 9:1 water-acetone solution and a 1-minute post-desorption wash in a 1:1 water-acetone solution. This cleaning ensured optimal extraction for up to 120 consecutive extractions in soya milk samples diluted in the ration of 1:1 with ultrapure water. To improve analyte recovery, various organic modifiers were tested and evaluated (Gionfriddo et al., 2020).

The increasing consumption of soya beverages has driven the optimisation of methods for pesticide determination, with approaches such as QuEChERS combined with UHPLC-MS proving suitable for routine laboratory analysis. Additional techniques, such as DLLME and GC with various detectors, have also been explored, emphasising

the importance of solvent selection and clean-up steps to avoid analytical interference. Innovative methods, such as DI-SPME with matrix-compatible fibres, show advancement in pesticide analysis for complex matrices such as soya milk.

Flour

Soya flour is a product made by grinding roasted or non-roasted soybeans into a fine powder. It is a versatile ingredient that can be used in a variety of culinary applications. Soya flour is rich in protein, dietary fibre, and other nutrients. Owing to the dry nature of this sample, special approach to sample extraction must be assumed.

For the detection of pesticides in soya bean flour, a solid-liquid extraction (SLE) method was employed, followed by HPLC with a diode array detector (HPLC-DAD). The extraction process relied on the differential affinities of sample components across three phases: aqueous solution, acetonitrile, and n-hexane. Initially, acetonitrile was added to the solid sample, facilitating the extraction of analytes along with other compounds, such as lipids. To purify the sample, an aqueous phase (NaOH solution), a cleaning solvent (n-hexane), and a phase-separation agent (NaCl) were introduced to the extract. The addition of NaCl resulted in a three-phase system where hydrophobic compounds were extracted into n-hexane, polar compounds into the aqueous phase, and the analytes remained in the acetonitrile phase. The extraction yielded efficiencies between 55 % and 90 % (Abbaspour et al., 2019b).

Pizzutti et al. (2007) developed a method using liquid chromatography-mass spectrometry (LC-MS) for pesticide determination in soya bean flour. Flour samples underwent acetone-based extraction without subsequent purification by sorbents. The method demonstrated excellent linearity for calibration curves in the range of 0.1 or 0.25 to 10.0 $ng \cdot mL^{-1}$, with LODs generally spanning from 0.1 to 0.25 $ng \cdot mL^{-1}$. Recovery rates ranged from 70 % to 120 %. This acetone-based extraction method proved reliable for quantifying significant pesticide residues in the complex matrix of soya bean rice, thus proving suitable for routine surveillance and research application (Pizzutti et al., 2007).

Soya bean rice is similar to soya flour concerning the composition and nature. For both soya bean rice and flour, LLE and acetone-based extraction methods were utilised. Post-extraction, liquid chromatography (LC) was employed to separate the analytes. The overall recovery rates for pesticides ranged from 55 % to 120 % across both methods, demonstrating the effectiveness of these extraction and analysis techniques in dealing with complex food matrices.

The use of LLE followed by HPLC-DAD and LC-MS methods has proved to be effective for the determination of pesticides in soya bean flour and rice. The extraction processes, optimised with different solvents and phase-separation techniques, ensure high recovery rates and accurate detection, making these methods suitable for both routine and detailed pesticide analysis in complex matrices.

Dietary supplements

Soya-based dietary supplements cover a wide range of products intended for direct consumption improving the health of the population. They may contain soya powder, rich in proteins, or they are based on extracts with a variable composition. Research on the determination of pesticides in these supplements primarily focuses on developing and validating analytical methods to ensure the safety and quality of these products. Pesticides, used in agriculture to protect crops from pests, can remain as residues in food products including soya-based supplements. Given the potential health risks associated with pesticide exposure, it is crucial to monitor and regulate these residues.

Páleníková et al. (2015) investigated the optimisation of pesticide extraction methods from soya dietary supplement samples using a modified QuEChERS approach. Their study evaluated various extraction solvents and purification procedures. The most effective conditions were identified using a combination of PSA, C18, GBC, and Zr-Sep+ sorbents with ethyl acetate as the solvent. This method consistently yielded reliable recoveries between 70 % and 120 % for 92 % of the pesticides studied. In contrast, the use of acetonitrile as the solvent resulted in reliable recoveries for only 28 % of the pesticides, which is likely connected to analyte loss during evaporation (Páleníková et al., 2015).

Alves et al. (2016) employed a modified rapid extraction method, quick polar pesticides method (QuPPe), coupled with liquid chromatography and mass spectrometry to determine polar pesticides in soya dietary supplements. Their study explored various experimental conditions, including solvent selection, oxygenation, extraction time, and cleaning sorbents. Acetonitrile acidified with formic acid was used as the extraction solvent, leading to a viscous mixture unsuitable for injection into the liquid chromatograph. Optimal separation was achieved using a hypercarb-porous graphitic carbon stationary phase. Pesticide recoveries ranged from 46 % to 119 % (Alves et al., 2016).

Soya-based infant foods were analysed by LC/ESI-MS/MS. The method includes liquid–liquid partitioning and solid-phase extraction (SPE) with Oasis HLB cartridges to remove soya proteins and

Tab. 1. Overview of sample analysis and preparation details of published analytical methods applied for soya products analysis.

Sample	Pesticides	Sample preparation	Clean up	Instrumentation	LOD (μg·kg^{-1} or μg·L^{-1})	Real sample findings	Ref.
soya bean	10 pesticides	QuEChERS	C18, PSA, Florisil	LC-MS	1—5	ND	Huerta-Peréz, 2015
soya bean	Glyphosate and 13 polar pesticides	SLE (methanol)	EMR-Lipid, CAPTIVA, WCX, HLB, MCX	HILIC LC-MS	0.02—0.05	ND	Herrera, 2019
soya bean	8 pesticides	SLE	PSA, C18	GC-IDMS	10—25	ND	Huerta-Pérez, 2019
soya bean	7 pesticides	QuEChERS	$MgSO_4$, PSA	CE-MS	less than 0.1	ND	Daniel, 2018
soya bean	25 pesticides	SLE	Bio-Beads S-X3	GC-MS	2—50	ND	Hong, 1993
soya bean	6 pesticides	MSPD	InertSep C8	HPLC	40—80 μg · g^{-1}	ND	Maldaner, 2008
soya bean	30 OPP	QuEChERS	PSA, DSC-18	GC-MS	0.2—3	Chlorpyriphos, Pirimiphos-methyl	Marchis, 2012
soya bean	257 pesticides and micotoxins	QuEChERS	Florisil	LC-MS	5—10	ND	Martínez-Domínquez, 2016
soya bean	203 pesticides	SLE (hexane, acetonitrile)	SPE Florisil	GC-MS	less than 10	ND	Shin, 2018
soya bean	10 pesticides	QuEChERS	DLLME (200 μL DES)	GC-MS	5—26	ND	Petrarca, 2024
soya bean oil	169 pesticides mixture	QuEChERS	PSA, Z-Sep, EMR-Lipid	UHPLC-QqQ-MS	0.01—0.05	Azoxystrobin <LOQ	Dias, 2016
soya bean oil, and other oils	group of 14 pyrethroid pesticides	SMDP	Florisil, Alumina	M-CS-EC	13—53	ND	Di Muccio, 1999
soya bean oil, nuts, sesame oil	28 pesticides	dSPE (PSA, C18)	Low temperature cleanup	GC-FPD, GC-MS	20—255	ND	Li 2007
soya bean oil	126 pesticides	LLE (hexane, acetonitrile)	SPE (Captiva EMR-Lipid)	GC-MS/MS	5—50	ND	Wang, 2021
soya milk	7 pesticides	LLE DLLME	PSA	DSPE	0.035—0.12	Chlopyriphos 19 μg · L^{-1}	Abbaspour, 2019
soya milk	group of triazolopyrimidine sulphonanilide pesticides	SPE	NaOH	CE-MS	less than 74	ND	Hernández, 2005
soya milk	8 sulphonylurea herbicides	QuEChERS	Z-Sep	HPLC-DAD, LC-MS	10—100	Chlorsulphuron 14.2 ng · mL^{-1}	Rejczak, 2016
soya milk	10 pesticides	DI-SPME (PDMS/DVB/PDMS)	–	GC-MS	1—2.5	Trifluralin Dimethoate Malathion Chlorpyrifos Thiabendazole λ-Cyhalothrin β-Cyfluthrin Esfenvalerate 0.05—8 μg · kg^{-1}	Gionfriddo 2020

Tab. 1. (continue) Overview of sample analysis and preparation details of published analytical methods applied for soya products analysis.

Sample	Pesticides	Sample preparation	Clean up	Instrumentation	LOD (μg·kg^{-1} or μg·L^{-1})	Real sample findings	Ref.
soya drink	group of 39 pesticides	QuEChERS d-SPE	C18, PSA	UHPLC-MS	10—25	ND	May, 2017
soya drink	strobilurin fungicides	DLLME	ND	LC-DAD-MS	0.8—4.5	ND	Campillo, 2015
soya rice	group of 169 pesticides	LLE	ND	LC-MS	0.001—0.025	ND	Pizzutti, 2007
soya flour	group of 9 pesticides	LLE LLME	C18, Florisil	HPLC-DAD	0.016—0.06	ND	Abbaspour, 2019
soya-based infant formula	13 pesticides	SLE (acetonitrile, hexane)	SPE (Oasis HLB Plus)	LC-MS/MS	0.1—0.6	ND	Wang, 2006
soya-based infant formula	glyphosate and AMPA	SLE (chloroform)	SPE	HPLC-FD	20	Glyphosate 0.03—1.08 mg · kg^{-1} AMPA 0.02—0.17 mg · kg^{-1}	Rodrigues, 2018
soya nutritional supplements	4 polar pesticides (Chlorate, Fosetyl-Al, Maleic Hydrazide, Perchlorate)	QuPPe	Hypercarb	LC-MS	4—100	Chlorate 63 to 1642 μg · kg^{-1}	Alves, 2016
soya nutritional supplements	group of 177 pesticides	QuEChERS	PSA, C18, GBC, Z-Sep	GC-QqQ-MS	0.1—10	Malathion 11.1 μg · kg^{-1}, Pyriproxyfen 1.5 μg · kg^{-1}	Páleníková, 2015

other interferences and isolate pesticides from the samples. Isoprocarb was used as the internal standard (IS), which helps compensate for pesticide losses and variations during extraction, mitigating matrix effects and ensuring accurate quantitative results. However, due to matrix effects and poor accuracy, only semiquantitative analyses should be done for propoxur and aldicarb (Wang et al., 2006). Overall, these studies highlight the importance of method optimisation and selection of appropriate extraction solvents and purification procedures for accurate pesticide analysis in soya dietary supplements to ensure consumer safety and regulatory compliance.

Real-life findings

The analysis of pesticide residues in soya and soya products is crucial for ensuring food safety and public health protection. Pesticide residues in soya products can pose health risks to consumers if present at levels exceeding regulatory limits or if they include pesticides known to be harmful to human health. Analysing real samples allows for the assessment of actual exposure risks and ensures that products on the market are safe for consumption.

Studies have identified various pesticides, including glyphosate and organo-chlorine pesticides. Glyphosate, in particular, is frequently detected due to its widespread use in soya bean cultivation. Although glyphosate is a universally used pesticide, monitoring has been hampered by analytical difficulties due to its high polarity. Kolakowski et al. (2020) examined 204 samples of soya-based products, including canned/frozen soya beans, soya beverages, and meat alternatives. The prevalence of glyphosate residues varied by product type, with no residues detected in canned soya beans and tofu, and up to 37 % in the analysed soya flour. The maximum glyphosate levels ranged from 0.013 ppm in frozen soya beans to 6.0 ppm in soya flour. These levels were significantly below the maximum residue limits. Soya flour consistently showed higher contaminant levels compared to other soya products (Kolakowski et al., 2020). Glyphosate and amino methyl phosphonic acid (AMPA) were detected in baby food formula by Rodrigues and Souza (2018), marking the first study to report glyphosate in such products. Their find-

ings showed a detection rate of 50.5 % for glyphosate and 19 % for AMPA, with mean concentrations of 0.19 $mg \cdot kg^{-1}$ and 0.05 $mg \cdot kg^{-1}$, respectively (Rodrigues et al., 2018).

Detection of polar pesticides like chlorate and perchlorate has also been investigated, as it requires specialised analytical methods due to the chemical properties of these compounds. Although chlorate has been banned in the EU since 2010, it was determined in five soya-based dietary supplements out of the fourteen analysed samples at high concentrations ranging from 63 to 1642 $\mu g \cdot kg^{-1}$ (Alves et al., 2016).

A high number of pesticides were detected in commercial samples of soya milk from two different brands (Gionfriddo et al., 2020). The concentration of dimethoate in one of the samples was higher than the MRL set for soya products (118.9 $\mu g \cdot kg^{-1}$). Overall, analysing real soya samples for pesticide findings is essential for safeguarding public health, ensuring regulatory compliance, maintaining product quality, and minimising environmental impact associated with pesticide use in soya bean production.

Future trends, challenges and conclusions

This manuscript highlights the growing significance of soya beans in vegetarian diet and their extensive use in various industries while emphasising the critical need to monitor pesticide residues in soya products for food safety. The increased demand for soya beans has led to greater pesticide use, posing risks to human health and the environment. Various analytical methods, particularly modifications of the QuEChERS method, have been developed and optimised to effectively extract pesticide residues in complex soya matrices. The review covers sample preparation and analysis of soya beans, soya oil, soya beverages, flour, and dietary supplements, demonstrating the efficacy of advanced chromatographic and mass spectrometric techniques. Ensuring the safety of soya products requires continual improvement and validation of these analytical methods to meet regulatory standards and protect consumers. The findings underscore the necessity for stringent monitoring and innovative approaches to managing pesticide residues in the face of increased soya bean consumption. Future trend is the development of analytical methods applicable to the detection and quantification of multiple groups of analytes in a single analysis with increased degree of sensitivity selectivity, as well as of faster, easier and solventless or solvent-minimised methods. Progress in procedures meeting the green analytical sample preparation and green analytical chemistry requirements is expected.

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