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Review

Determination of pesticide residues in fruit and vegetables

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Abstract

A review concerning the determination of pesticide residues in fruit and vegetables is presented. The basic principles and recent developments in the extraction and quantitation of pesticides are discussed. Consideration is given to solid phase and supercritical extraction techniques, automation and robotic systems, and immunoassay procedures.

Keywords: Food analysis; Reviews; Fruits; Vegetables; Extraction methods; Environmental analysis; Pesticides

Contents

1. Introduction

The use of pesticides provides unquestionable benefits in increasing agricultural production. However, it has the drawback of pesticide residues which remain on fruit and vegetables, constituting a potential risk to consumers [1]. This stimulates on one hand, the establishment of legal directives to control their levels through the Maximum Residue Levels (MRLs), and on the other, a continuous look for

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pesticides less persistent and toxic for the human being. This fact has increased extraordinarily the number of pesticides registered and/or recommended, and analytical difficulties for their control [2,3].

To detect agricultural products that contain pesticide residue levels higher than the MRLs each country has available governmental agencies, which monitor pesticide residues through two different but complementary approaches: regulatory monitoring focused on raw agricultural commodities which measures the levels in individual lots for determining compliance with legal tolerances [4–11], and the Total Diet Study, in which dietary intakes of pesticides are determined by analysis of fruit and vegetables as consumed [11–15].

Analytical methods are needed to screen, quantify, and confirm pesticide residues in fruit and vegetables for both research and regulatory purposes. Multiresidue methods (MRMs) and single residue methods (SRMs) generally consist of the same basic steps, but the first ones are preferred to the second ones for the analysis of pesticides, because MRMs provide the capability of determining different pesticide residues in a single analysis. A review of the methods currently used to extract, isolate, and quantify pesticide residues in fruit and vegetables by monitoring agencies, demonstrates that they are based on classical MRMs, some developed over 30 years ago. Among the more widely used MRMs are those of Mills [16]; Mills, Onley, and Gaither [17]; Storherr [18]; Luke [19]; and Krause [20].

The method of the Association of Official Analytical Chemists (AOAC), typifies the international recognized MRMs [21]. It allows the determination of many pesticide residues in fruit and vegetables, and involves an aqueous acetone extraction and laborious cleanup. Such methods, generally, applied an extraction step with a water miscible solvent, followed by a cleanup step, with an organic solvent of limited water capacity, to achieve the removal of interferences present in the sample extract and/or solid phase cleanup with silica or florisil. Finally, analyte determination is performed by gas chromatography (GC) or high-performance liquid chromatography (HPLC) with selective detectors [22,23].

These methods detect approximately 325 pesticide and pesticide-related compounds and most of them have undergone rigorous multilaboratory calibration studies, such as those needed to obtain the official acceptance by the AOAC [24]. However, their continued use still presents disadvantages, such as (i) their inefficiency as screening methods: These methods are too complex, and they do not allow the generation of relevant data in time to prevent contaminated foods from entering the marketplace, because these procedures are time consuming and labour intensive; (ii) the amount of chemicals and toxic solvents that are used: it is usually by a factor of 10^8–10^{10} greater than that of the pesticide residues to be determined; (iii) in addition, the newly developed groups of pesticides are each time more polar and/or thermodegradable and they should be incorporated into the existing MRMs.

To avoid the general drawbacks of the classical methods, in recent years, significant evolution was noted in the extraction and determination of pesticide residue analysis in fruit and vegetables [25–27]. This review tries to cover the literature about the above mentioned progress published in the last 10 years for the pesticide residue analysis. The main attention is paid to simplification, miniaturization, and improvement of sample extraction and cleanup methods with universal microextraction procedures, solid-phase extraction (SPE) and/or solid-phase cleanup (SPC) on cartridges to replace liquid–liquid extraction (LLE), matrix solid-phase dispersion (MSPD) and selective extraction with supercritical fluid (SFE). Determination of the pesticide residues, by GC using microwave induced plasma (MIP)-atomic emission detector (AED), and tandem mass spectrometry (MS–MS), and by HPLC, for thermally labile and/or polar pesticides and metabolites, using ion and ion-pair chromatography, additional postcolumn derivatization techniques and improvement of the HPLC detectors, are discussed. In addition, supercritical fluid chromatography (SFC) with different modified supercritical fluids and improved detectors for the analysis of nonpolar and polar analytes and the on-line combination SFE–SFC, are also reported. Some attention is given to the development of reliable enzyme immunoassay procedures for pesticides and metabolites, specially in the areas of sample preparation, validation, multiresidue capa-

bility and commercial kits and biosensors, which generally involve an immobilized enzyme or antibody as the basis of selectivity.

2. Pesticide residues and legislation

As has been previously commented, since 1960's fresh fruits and vegetables have been checked for pesticide residues [4,11]. Nowadays the number of pesticides that could be detected number over 380. About 99 of them are actually found [9,11]. These pesticides present a wide variety of uses and physico-chemical properties. In this way it is common to equate pesticides with insecticides. This is erroneous since the term pesticide is a general classification and includes mainly insecticides, herbicides and fungicides. Each group of compounds includes different chemical families and types of action, and also, one compound may present a diversity of uses. Table 1 listed the pesticide residues found in fresh fruits an vegetables by the official agencies during 1992–1993 in pesticide residues, their uses and chemical class.

The levels of pesticide residues are controlled by the MRLs, which are established by each country and sometimes cause conflicts because residue levels acceptable in one country could be unacceptable in other. This problem has revealed the need to harmonize the different MRLs, which has mainly been dealt with by two international organizations: the European Union (EU) at European level and the Codes Alimentarius Commission of the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) [2,28].

The first EU directive was promulgated in 1976 (Directive 76/895/EEC) and it fixed the MRLs of certain pesticides in/on fruits and vegetables. This directive was modified and extended in later directives, published in 1980 (80/428/EEC), 1981 (81/36/EEC), 1982 (82/528/EEC), 1988 (88/298/EEC) and 1989 (89/186/EEC). The main disadvantage associated with these directives is that they only give partial harmonization because they do not cover all the pesticides traded, since they only reach to 64 active ingredients. National legislations cover more: about 380 in Spain, 360 in Germany, 400 in Netherlands, etc. At world level, there are more than 600 active ingredients in the market [29].

In 1990, the EU promulgated a new directive (90/642/EEC) relating to the MRLs in selected plant products, including fruits and vegetables. Its object is to avoid the diversity of MRLs in order to facilitate the future European one-trade system. It fixes the MRLs for all the EU countries, eliminating the possibility that some countries approve higher MRLs. As a result, the products with residue contents higher than MRLs established by the EU can not be moved between the member countries [2,28].

Moreover, there are directives relating to the ban of marketing and use of some OCPs. They started with directive 79/119/EEC and included 83/131/EU, 85/298/UE, 86/355/EU, 87/181/EU, 87/477/EU, 89/365/EU, 90/533/EU. The directive 79/100/EEC establishes sampling methods for the official control of pesticide residues in fruits and vegetables, and 85/591/EEC treats the introduction of sampling and analysis methods to control products for human consumption. Recently, a directive 91/414/EU about the marketing of pesticide products was published. It demands a large number of studies on residues before an active ingredient can be authorized at European level [28].

3. Extraction and clean-up

3.1. Liquid–liquid extraction (LLE)

The existing multiresidue methodology makes possible the determination of OCPs, OPPs, MCs, triazine and thiocarbamate herbicides, Dithiocarbamates, and other contaminants in crops. These MRMs are continuously being revised to reduce their disadvantages. It is possible to diminish the following drawbacks:

1. Toxicity of solvents used
2. Partition step
3. Column cleanup
4. Incorporation of the newly developed pesticides

Table 1
Pesticides, isomers and breakdown products that have been detected in fresh fruits and vegetables

Compound	Use	Chemical class	References
Acephate	Insecticide	OPPs[a]	[9,11]
Aldicarb	Insecticide	MCs[b]	[11]
Aldicarb sulfone	Insecticide	MCs[b]	[11]
Aldrin	Insecticide	OCPs[c]	[11]
Anilazine	Herbicide	Triazine	[11]
Azinphos-ethyl	Insecticide	OPPs	[9]
Azinphos-methyl	Insecticide	OPPs	[9,11]
Bitertanol	Fungicide	Nitrogen Heterocyclic	[9]
Bromide (inorganic)	–	–	[9]
Bromopropylate	Acaricide	Bromo Benzylate	[9]
Bupirimate	Fungicide	Nitrogen Heterocyclic	[9]
Captafol	Fungicide	Dicarboximide	[9,11]
Captan	Fungicide	Dicarboximide	[9,11]
Carbaryl	Insecticide	MCs	[9,11]
Carbendazim	Insecticide	MCs	[9,11]
Carbofuran	Insecticide	MCs	[11]
Carbophenothion	Insecticide	OPPs	[11]
Chlordane	Insecticide	OCPs	[11]
Chlordimeform	Insecticide	Formamide	[11]
Chlorfenvinphos	Insecticide	OPPs	[9,11]
Chlorobenzilate	Acaride	Chloro Benzylate	[9]
Chlorothalonil	Fungicide	Nitrogen heterocyclic	[9,11]
Chlorpropham	Herbicide	Carbamate	[9]
Chlorpyrifos	Insecticide	OPPs	[9,11]
Chlorpyrifos-methyl	Insecticide	OPPs	[9,11]
Chlozolinate	Fungicide	Nitrogen Heterocyclic	[9]
Cypermerthrin	Fungicide	Pyrethrine	[9,11]
Daminozide	Grow regulator	Hydrazide	[9]
DCPA	Herbicide	Chlorophenoxy	[11]
p,p'-DDE	Insecticide	OCPs	[9]
DDT	Insecticide	OCPs	[11]
Deltamethrin	Fungicide	Pyrethrine	[9]
Demeton	Insecticide	OPPs	[11]
Diazinon	Insecticide	OPPs	[9,11]
Dichlobenil	Herbicide	Nitriles	[11]
Dichlofluanid	Fungicide	Nitrogen Heterocyclic	[9]
Dichlorvos	Insecticide	OPPs	[9,11]
Dicloran	Fungicide	Sustituted Aromatic	[9,11]
Dicofol	Insecticide	OCPs	[9,11]
Dicrotophos	Insecticide	OPPs	[11]
Dieldrin	Insecticide	OCPs	[9,11]
Dimethoate	Insecticide	OPPs	[9,11]
Diphenylamine	Other treatments	–	[9,11]
Diquat	Herbicide	Bipyridyl	[9]
Dithianon	Fungicide	Nitrogen Heterocyclic	[9]
Disulphoton	Insecticide	OPPs	[11]
Endosulfan-alfa	Insecticide	OCPs	[9,11]
Endosulfan-beta	Insecticide	OCPs	[9,11]
Endosulfan sulphate	Insecticide	OCPs	[9,11]
Endrin	Insecticide	OCPs	[9,11]
EPN	Insecticide	OPPs	[11]
Esfenvalerate	Insecticide	Pyrethrine	[11]

Table 1 (*continued*)

Compound	Use	Chemical class	References
Ethion	Insecticide	OPPs	[9]
Ethoprop	Insecticide	OPPs	[11]
Etrimfos	Insecticide	OPPs	[9]
Fenarimol	Fungicide	Nitrogen Heterocyclic	[9]
Fenitrothion	Insecticide	OPPs	[9,11]
Fenthion	Insecticide	OPPs	[9,11]
Fenthion sulphone	Insecticide	OPPs	[9]
Fenthion sulphoxide	Insecticide	OPPs	[9]
Fenvalerate	Insecticide	Pyrethrine	[9]
Folpet	Fungicide	Dicarboximide	[9,11]
Fonofos	Insecticide	OPPs	[11]
Heptachlor	Insecticide	OCPs	[11]
Heptachlorobenzene	Insecticide	OCPs	[11]
γ-HCH	Insecticide	OCPs	[9,11]
Imazalil	Fungicide	Nitrogen Heterocylic	[9,11]
Iprodione	Fungicide	Nitrogen Heterocyclic	[9,11]
Linuron	Herbicide	substituted Ureas	[11]
Malathion	Insecticide	OPPs	[9,11]
Mancozeb	Fungicide	Dithiocarbamates	[9]
Maneb	Fungicide	Dithiocarbamates	[9]
Mecarbam	Insecticide	MCs	[9,11]
Metalaxyl	Fungicide	Nitrogen Heterocyclic	[9]
Methamidophos	Insecticide	OPPs	[9,11]
Methidathion	Insecticide	OPPs	[9,11]
Methiocarb	Insecticide	MCs	[9,11]
Methomyl	Herbicide	Carbamate	[11]
Metribuzin	Herbicide	Triazine	[9]
Mevinphos	Insecticide	OPPs	[9,11]
Mirex	Insecticide	OCPs	[11]
Monocrotophos	Insecticide	OPPs	[9,11]
Myclobutanil	Fungicide	Nitrogen Heterocyclic	[11]
1-Naphtol	–		[9]
Omethoate	Insecticide	OPPs	[9,11]
Ortophenylphenol	Fungicide	Substituted aromatics	[9]
Oxadiazon	Insecticide	OPPs	[11]
Oxamyl	Herbicide	Carbamate	[11]
Parathion	Insecticide	OPPs	[9,11]
Parathion-methyl	Insecticide	OPPs	[9,11]
Penconazole	Fungicide	Nitrogen Heterocyclic	[9]
Pentachloroanisole	Fungicide	Substituted aromatics	[9]
Permethrin	Insecticide	Pyrethrine	[9,11]
Phentoate	Insecticide	OPPs	[9]
Phorate	Insecticide	OPPs	[11]
Phosalone	Insecticide	OPPs	[9,11]
Phosmet	Insecticide	OPPs	[9,11]
Phosphamidon	Insecticide	OPPs	[9,11]
Pirimicarb	Insecticide	MCs	[9]
Pirimiphos-methyl	Insecticide	OPPs	[9,11]
Prochloraz	Fungicide	Nitrogen Heterocyclic	[9]
Procymidone	Fungicide	Dicarboximide	[9,11]
Profenofos	Insecticide	OPPs	[11]
Pronamide	Herbicide	Amide	[11]
Propargite	Acaricide	Sulphite	[9,11]

(*Continued on p. 306*)

Table 1 (*continued*)

Compound	Use	Chemical class	References
Propham	Herbicide	Carbamate	[9]
Propineb	Fungicide	Dithiocarbamate	[9]
Propyzamide	Herbicide	Amide	[9]
Prothiofos	Insecticide	Insecticide	[9]
Quinalphos	Insecticide	Insecticide	[9,11]
Quintozene	Fungicide	Substituted Aromatics	[11]
Sulfotep	Insecticide	OPPs	[9,11]
Sulphur dioxide	–	–	[11]
2,3,5,6-TCA	Herbicide	Chlorophenoxy	[9]
TDE	Insecticide	OCPs	[11]
Tecnazene	Fungicide	Substituted Aromatics	[9]
Terbufos	Insecticide	OPPs	[11]
Tetradifon	Insecticide	OPPs	[9,11]
Thiabendazole	Fungicide	Nitrogen Heterocyclic	[9,11]
Thiram	Fungicide	Dithiocarbamate	[9]
Tolclofos-methyl	Insecticide	OPPs	[9]
Tolyfluanid	Fungicide	Substituted Aromatics	[9]
Toxaphene	Insecticide	OCPs	[11]
Triadimefon	Fungicide	Nitrogen Heterocyclic	[9,11]
Triadimenol	Fungicide	Nitrogen Heterocyclic	[11]
Tri-allate	Herbicide	Carbamate	[11]
Triazophos	Insecticide	OPPs	[9]
Trichlorfon	Insecticide	OPPs	[9]
Vinclozolin	Fungicide	Nitrogen Heterocyclic	[9,11]
Zineb	Fungicide	Dithiocarbamate	[9]

[a] OPPs: Organophosphorus Pesticides.
[b] MCs: Methyl Carbamates.
[c] OCPs: Organochlorine Pesticides.

3.1.1. Diminution of the organic solvent toxicity

In this way, even the AOAC method, which is one of the most commonly instituted methods, has been modified. The original method, that with extraction by acetonitrile, followed by liquid–liquid partitioning with petroleum ether–dichloromethane and a laborious Florisil column cleanup, was modified in 1985 to include acetone instead of acetonitrile [21]. It has also been incorporated in recent revisions of the Pesticide Analytical Manual (PAM) [30]. Most of the current FDA analytes are screened by this extraction scheme [11].

Acetone extraction was usually preferred since it is suitable for both non-polar and polar pesticides [31,32], as has been demonstrated in different comparative studies performed by GC and HPLC [33,34]. Acetone has low toxicity, is easy to purify, evaporate and filter, and is cheap. Fruit and vegetable extracts in acetone are usually cleaner than those obtained with other solvents of similar polarity.

The National Food Administration of Sweden [35], also used acetone extraction followed by partitioning with hexane–dichloromethane, and twice with dichloromethane. After the cleanup method on an SX-3 permeation chromatography column, residues are determined by GC using ECD, NPD, FPD and FID.

In Germany, pesticide analysis in fruit and vegetables is mainly performed with the MRM S19 of the Deutsche Forschungsgeneinschaft (DFG) pesticide commission. This method was developed to obtain extracts suitable for GC determination with selective detectors, mainly ECD, NPD, and FPD. It extracts with acetone–dichloromethane, and pesticide residues are detected after the cleanup by gel permeation chromatography (GPC) and mini-silica gel column fractionation in up to six fractions. The data about elution and recoveries of more than 400 pesticides, their metabolites and a few common pollutants are well documented [36,37].

According to the multiresidue analysis method DFG S19, different approaches for the substitution of dichloromethane in the liquid–liquid partition (LLP) step were investigated. The comparison of pesticide recoveries show that several less toxic solvents like cyclohexane, light petroleum and tertiary butyl methyl ether are suitable substitutes for the extremely toxic dichloromethane [38].

3.1.2. Elimination of the partition step

A rapid and efficient multiresidue extraction procedure using ethyl acetate and sodium sulphate, followed by GPC on an SX-3 column, was first reported by Roos et al. [39]. Recoveries better than 90% were obtained for OCPs and OPPs, fungicides and chlorobiphenyls. Since July 1989, this method is also being used by the National Food Administration of Sweden as general MRM [40], replacing the MRM proposed by Anderson and Ohlin [35]. The number of pesticides, isomers and breakdown products that can be detected number about 160.

The ethyl acetate and sodium sulphate extraction without further cleanup was applied as screening method for the analysis of eight OPPs with different polarities in different kinds of vegetables using GC–FPD and GC–NPD. With the use of specific detectors, interfering chromatographic peaks were decreased and the analysis time and solvent were reduced, resulting in cheaper analyses [41–43]. Gas chromatography with mass spectrometry (GC–MS) was also used for determination in crop samples with less than 2% fat [44]. However, when ECD or FID were employed, the extracts presented serious interference problems. In these cases, some authors proposed cleanup method simpler than classical GPC, such as the employ of silica gel cartridge for OC and pyrethroid pesticides [45] or the use of an on-line LC–GC method, consisting of a silica HPLC column, which provided the separation between fenarimol and matrix components and its direct introduction into the GC via the loop type interface technique [46].

The ethyl acetate methods are also called on-line extraction methods because they omit a separate LLP. The theoretical principle of the on-line method is presented by the Gibbs triangle. Other solvents can be used in these on-line approaches such as hexane–acetone mixture (8:2) [38], ethyl acetate–xylene [47], acetone or acetonitrile–dichloromethane or petroleum ether [48]. Also the use of dichloromethane followed by cleanup over silica gel for the determination of nitrogen-containing pesticides has been systematically studied [49].

Previously, it was difficult to miniaturize any of the conventional extraction methods without great difficulties. However, the on-line methods can be miniaturized very easily, so that the solvent consumption is reduced to 1/10–1/100 of the original amount. The microtechniques have been validated by the analysis of OPPs in fruit and vegetables with unknown history [48,50–52].

A collaborative study for the determination of OPPs in fortified samples of lettuce and pears was conducted by comparison with the results of the macro on-line method, the macro off-line method and the Soxhlet extraction method. The results of the micromethod compared well with those of the macromethods. The average recoveries of the micromethod ranged from 88 to 107%, and those of the macro methods from 80 to 107% [53].

To replace classical LLP, and to reduce analysis costs and pollution, an SPE method has been developed. In this process, the compound is isolated from a liquid sample by differences in the relative solubilities between a liquid mobile phase and a stationary phase.

The California Department of Food and Agriculture (CDFA) uses a modified Mill's method, consisting of acetonitrile extraction and cleanup on C_{18} [54]. The pH of the filtrate is adjusted to neutral with phosphate, and the acetonitrile layer is separated from the aqueous layer by a salting out process. This method was evaluated by analysing for seven OCPs, seven OPPs and seven MCs at 0.1–0.2 μg/g in six representative fruits and vegetables using GC and HPLC.

Consalter and Guzzo [55] effected the cleanup using Sep-Pack C_{18} and Bond Elut 2 OH cartridges by the salting out effect. The results proved that it is possible to apply them to the determination of OPPs in crops.

The feasibility of the solid-phase to substitute the LLP was examined using seven different reversed bonded-phase silica sorbents (C_{18}, C_8, C_2, C_1, CH, PH, CN) [56]. The C_{18} showed acceptable recoveries for almost all the OPPs and MCs tested, and its

applicability to a cleanup of crude sample extracts from crops. C_{18} and Florisil cartridges were evaluated for the cleanup of crop matrices extracted with acetonitrile for organohalogen pesticides [57]. Cleanup with Attagel was also included for comparison. Cleanup with either C_{18} or Florisil showed recoveries comparable to, or better than that obtained with Attagel. SPE cartridges, containing normal or reversed-phase supports, have become available commercially and offer the potential of simplifying the purification of the initial extract as well as reducing the amount of solvent consumed. C_{18} commercial cartridges were examined for the cleanup of crop extracts in the determination of fungicide [58] and OCPs [59].

Column extraction using diatomaceous earth (ca. 40 g) as adsorbent was introduced as an alternative to the LLP step in the Luke procedure [60]. Recoveries from the column are quantitative and reproducible for a wide range of polar and nonpolar OPPs. The same column was checked to replace the LLP step for the methods used in the determination of bitertanol, dichlofluanid, tolylfluanid and tebuconazole [61]. The recoveries were in the range of 77–110%, and the routine limit of determination in plant material was 0.02 mg/kg for bitertanol and 0.05 mg/kg for each of the other compounds. Disposable Extrelux-20 cartridges were used as support to carry out the extraction and clean-up of OPPs [62], and fungicides [63] from crude acetone extracts of vegetable products.

3.1.3. Elimination of the column cleanup

The first approach to reducing the column cleanup step was the employment of short Florisil columns [64,65] instead of the classical big ones. Other solutions adopted to solve this problem was the suppression of the cleanup step. For example, a method of multiresidue analysis of 48 pesticides allowed in Japan, was systematically developed based on capillary GC. Pesticides were simultaneously extracted from vegetable and fruit samples with acetone, or with acetonitrile from lipid-containing crops, and then reextracted into ethyl acetate. Column chromatography was not necessary for the quantitation of OPPs. However, the quantitation of OCPs and pyrethroid pesticides could not be conducted without cleanup, and thus, Florisil column chromatography was performed. Cleanup by silica gel column chromatography was necessary for carbamates [66].

Another proposed solution is the employment of a coagulation method. A multiresidue method for 23 OPPs in fruits and vegetables, consists of extraction with acetone, cleanup by coagulating solution (phosphoric acid and ammonium chloride) and reextraction with benzene [67]. This method is not suitable for the determination of polar pesticides, such as mevinphos and phosphamidon, and water insoluble pesticides, as crufomate and carbophenothion.

Moreover, it is not acceptable for crops that are rich in fat, such as soybean, and uses benzene as an extraction solvent which is forbidden in most countries due to its carcinogenic effects on human beings. A simplified method is described for determining seven OPPs in citrus fruit, banana, soybeans and wheat [68]. An analytical method for natural pyrethrines and 12 synthetic pyrethroids based on the addition of a coagulating solution was also developed [69]. In both methods, residues were extracted with acetonitrile or acetone, and if necessary, were partitioned into *n*-hexane. Coextractives were coagulated with a solution containing phosphoric acid and ammonium chloride.

A method widely used for the cleanup of pesticides is GPC. It has been used for sample cleanup in pesticide analysis since the early 1970s. Bio-Beads SX-3, a polystyrene type gel, has been used with solvents such as ethyl acetate, cyclohexane, toluene, or mixture of these [8,9,35,39,40,70–74]. Lunardi and Passini [75] described a cleanup procedure using a Waters Ultrastyrogel 500 A column with toluene. All these GPC techniques, using large columns and low flow-rates, need long analysis times and large amounts of solvents.

Grob and Kalin described on-line GPC–GC for the determination of chlorinated pesticides in lettuce using small size-exclusion chromatography columns [76]. The method allows automated integration of the sample preparation into the GC analysis and eliminates corresponding manual work. However, De Paoli et al. found this system unsatisfactory for the determination of OPPs in fruit because of interfering peaks in GC [77]. A liquid chromatographic step on silica gel was therefore inserted between the GPC and the GC steps to filter out polar by products.

Samples of fruits (apples, grapes and kiwi fruits) were extracted, then the extract, filtered or centrifuged, was injected into an automated on-line GPC–LC–GC–FPD. Recoveries were about 95% and the detection limits about 1 ng/g.

3.1.4. Incorporation of the newly developed pesticides

MRMs that include a smaller rage of pesticides, like carbamates and fungicides have been developed. *N*-methyl carbamates can be extracted using the Luke's procedure described previously in combination with a normal-phase aminopropyl bonded silica SPE column cleanup and LC postcolumn fluorogenic determination [78,79]. De Kok et al. [80] performed the cleanup method using an automated SPE cleanup apparatus. The cleaned-up extract is injected on-line into the LC carbamate analysis system. The dithiocarbamates are treated with tin (II)-chloride and determined quantitatively as carbon disulfide [81].

Triazine herbicides and their metabolites are extracted with methanol and the resulting coextractives are removed using solvent partition and cation-exchange solid-phase extraction chromatography [82].

Fungicides (mainly pyrethrin) were extracted with methanol, partitioned into nonmiscible water solvent and purified by column chromatography on sodium sulphate/Florisil/Celite/charcoal [83–85]. Moreover, Extrelux cartridges were tested as a cleanup step in the determination of benzimidazolic fungicides such as carbendazim and thiabendazole [86]. After extraction and cyclization of thiophanate methyl into carbendazim, the conversion of benomyl into carbendazim is carried out by adsorbing the raw extract onto the cartridges and percolating 0.1 *M* HCl through it. Benzimidazolic residues are partitioned into the acid solution whereas most of the co-extractives are retained on the column. The final clean-up is performed on a strong cation-exchange cartridge.

Triclopyr (3,5,6-trichloro-2-piridoxyacetic acid) was extracted from the matrices and derivatized separately to 2-chloroethylene ester with 2-chloroethanol-BCl_3 and methyl ester with diazomethane. The esters were then quantitated by GC–ECD and GC–NPD [87].

Diclofop methyl and its metabolite diclofop were extracted with acetone–light petroleum, were concentrated (diclofop was derivatized to its pentafluorobenzyl derivative), and then the products were purified on a chromatographic column containing alumina, silver-alumina and Florisil. Finally, they were detected by GC–ECD [88].

Glyphosate and aminomethylphosphonic acid were extracted from a crop with chlorhydric or acetic acid followed by a cation-exchange column cleanup and reaction with a heptafluorobutanol and trifluoroacetic anhydride. Derivatized analytes were quantified using GC–MS [89].

Diquat and Paraquat were extracted with acid solution, and then isolated from the digest using pH-controlled silica solid-phase extraction [90–94] or cationic resin [95]. They are usually determined by HPLC but can also be determined by GC via their hydrogenation with sodium borohydride–nickel(II) chloride [96]. Mepiquat chloride was extracted by a method based on ion chromatography [97]. The limit of determination is 0.05 mg/kg.

Formethanate was determined by blending with acidified acetonitrile. A sample was loaded onto a strong cation-exchange (SCX) SPE, which replace the injection loop of the LC injection valve [98].

3.2. Matrix solid-phase dispersion (MSPD)

MSPD is a new extraction and clean-up technique, that has been developed to avoid the general drawbacks of the LLE, such as the use of large amounts of solvent, the occurrence of troublesome emulsions with certain fruit or vegetable matrices, and their slowness [99].

The mechanism involved in MSPD appears to encompass sample homogenization and cellular disruption, exhaustive extraction, fractionation, and purification in a single process. Elution of the MSPD column with a solvent or a solvent sequence can provide a high resolution fractionation of target analytes that can be further purified by simultaneous use of co-columns of florisil. Polar materials such as chlorophylls, triglycerides and phytosterols, which are the common components in fruit and vegetables, are associated with the surface of florisil. For this reason, the final eluate can, in most cases, be directly analyzed or further concentrated or manipulated to meet the demands of the individual analysis [100].

Kadenski et al. [101] demonstrated the applicability of MSPD to a large number of fruit and vegetable matrices and pesticide residues. In most cases, samples were added with distilled water, if necessary, for proper blending. Plant material was mixed with florisil and, after that, extracted with methylene chloride–acetone or ethyl acetate. Ling and Huang [102] applied the same methodology to the determination of synthetic pyrethroid pesticide residues in vegetables. Table 2 shows the matrices and pesticides tested by this technique, the analytical performance of the method and the MRLs established by the European Union.

Stattford and Lin [103] described an MSPD methodology for measuring oxamyl and methomyl residues in apples and orange fruits using C_{18}, previously washed with hexane, methylene chlorhide, ethyl acetate and methanol. The homogeneized sample (10–15 g) is placed into a column, and after being washed with hexane, is eluted with 10 ml of methylene chloride. The eluent is dried under a stream of nitrogen before being injected into an HPLC with fluorescence detection. Torres et al. [104] explored the possibility of using MSPD for the determination of organochlorine and organophosphorus pesticide residues in oranges using different solid-phases (C_{18}, C_8, C_2, CN, silica, florisil and alumina). In order to achieve the recoveries obtained, the optimized method was applied to the determination of these pesticide residues in different fruit and vegetable samples [105]. The eluent obtained is dried to a volume of 0.5 ml under a stream of nitrogen, before being analyzed by GC with ECD, NPD, FPD or MSD. Table 3 shows the matrices and pesticides tested by this technique, the analytical performance and the MRLs established by the European Union.

This method constitutes a significant advance in simplicity and efficiency that makes it possible to screen more samples. The pesticides extracted represent a diversity of molecular structures and polarity characteristics. The three main advantages of MSPD are: (i) it permits rapid sample turnover, enhancing access to timely data on residue levels present in the sample; (ii) because it requires a small sample size, it decreases considerably the amount of solvent used compared to the classical methods and, thus, in turn, decreases environmental contamination and increases worker safety; (iii) it is suitable for robotic automation.

3.3. Supercritical fluid extraction (SFE)

Recently, SFE is being recognized, in the field of pesticide residue analysis, as a potential alternative to the classical solvent-based extraction and clean-up methods [106]. The application of the SFE has been demonstrated for a number of pesticides and/or metabolites from fruit and vegetable matrices [107–114]. It is summarized in Table 4.

Compared to the conventional solvent extraction methods for isolating pesticide residues from this kind of matrix, SFE offers a number of potential advantages, it obviates the use of organic solvents, improves extraction selectivity, reduces time, space and glassware, and it enables automation [112]. An additional advantage is that SFE can be coupled with solid-phase sorbents such as glass beads, alumina or octadecylsilane (ODS), and then extraction and clean-up of the sample occur in a single step, and the extracts are cleaner than with solvent based methods [107,110,113]. SFE also offers the possibility of the direct introduction of the extracts obtained into an SFC system [108].

SFE with CO_2 modified by methanol, is being increasingly employed for the extraction of pesticide residues of different polarity and physico-chemical properties. In Table 4, OCPs [113], OPPs [113,114], pentachloronitrobenzene [110], carbamate [107,109,113] and pyrethoid compounds [108,113] extracted from fruit and vegetable matrices are reported. In most cases, the residues were analyzed by off-line GC, HPLC with specific detectors, or GC–MS after SFE.

Lehotay et al. [110], demonstrated in a first study, that the extraction of various pentachloronitrobenzene pesticides from vegetables by SFE was clean enhough for direct injection to GC–MS in EI mode. The selection of the appropriate SFE conditions such as CO_2 density, temperature modifier, type of solid-phase used for trapping the analytes, and elution solvent can be manipulated to overcome most chromatographic interferences. In a later work, the same authors used an SFE multiresidue method for the determination of 46 pesticides of different polarity and physico-chemical properties from fruit and veg-

Table 2
Determination of pesticide residues in fruits and vegetables by MSPD with Florisil

Matrix	Pesticide	Recovery (%)	Concentration range ($\mu g\ l^{-1}$)	LD ($mg\ kg^{-1}$)	MRLs ($mg\ kg^{-1}$)	Reference
	Triazines (Herbicides)					
Cherries, Grapes	Ametryne	90–95	0.1–1	0.02	0.05	[101]
Melons, Pepper	Atrazine	87–100	0.05–0.2	0.02	0.01–0.1	
Plums, Potatoes	Metribuzin	82–95	0.1–1	0.02	0.1	
Raspberries	Prometryn	86–98	0.05–2.0	0.02	0.05–0.1	
Tomatoes	Secbumeton	91–102	0.05–1.0	0.01	0.05	
Apples, Apricots	Terbumeton	91–99	0.1–1.0	0.01	0.02–0.05	
Bananas	Terbutyl-azine	83–96	0.1–2.0	0.02	0.05	
Broccoli	Terbutryn	91–101	0.1–2.0	0.02	0.05	
Cucumbers						
Currants	*Carbamates (Insecticides)*					
Eggplant	Benthiocarb	80–95	0.2–2.0	0.1	–	[101]
Lemons,	Carbaryl	92–98	0.2–2.0	0.08	1.0–5.0	
Oranges	Carbofuran	90–101	0.2–2.0	0.08	0.1–2.0	
Pears, Radish	Carbosulfan	85–92	0.2–2.0	0.08	0.1–2.0	
Beets	Dioxacarb	87–96	0.1–2.0	0.02	–	
Brussels sprouts	Molinate	78–93	0.2–2.0	0.05	0.01	
Carrots, Celery	Pirimicarb	79–91	0.1–2.0	0.05	0.05–0.5	
Green beans						
Green peas	*Carbamates (Herbicides)*					
Kohlrabi	Cycloate	82–99	0.05–5.0	0.05	0.05	[101]
Lettuce	EPTC	90–94	0.1–0.5	0.04	0.05	
	Vernolate	84–97	0.1–1.0	0.05	0.05	
	OCPs (Insecticides)					
	Aldrin	92–102	0.01–0.1	0.0006	0.01	[101]
	o,p'-DDD	85–100	0.01–0.1	0.002	0.05	
	o,p'-DDT	83–103	0.01–0.1	0.002	0.05	
	p,p'-DDD	82–102	0.01–0.1	0.002	0.05	
	p,p'-DDE	83–99	0.01–0.1	0.002	0.05	
	p,p'-DDT	86–101	0.01–0.1	0.002	0.05	
	Dieldrin	87–99	0.01–0.1	0.0006	0.01	
	Endosulfan	87–105	0.01–0.1	0.001	0.2–1.0	
	HCB	83–96	0.01–0.1	0.0002	–	
	α-HCH	86–94	0.01–0.1	0.0004	0.02	
	β-HCH	87–95	0.01–0.1	0.001	0.01	
	Heptachlor	83–90	0.01–0.1	0.001	0.01	
	Heptachlor expoxide	80–94	0.01–0.1	0.001	0.01	
	Lindane	86–99	0.01–0.2	0.0004	0.1–1.0	
	OPPs (Insecticides)					
	Azinphos-methyl	82–89	0.05–1.0	0.007	0.5–2.0	[101]
	Bromophos	87–100	0.05–1.0	0.004	0.05	
	Chlorfenvinphos	80–89	0.05–1.0	0.01	0.5–1.0	
	Chlorpyriphos	90–98	0.05–2.0	0.002	0.05–0.3	
	Dialifos	79–88	0.1–2.0	0.01	0.01–3.0	

(*Continued on p. 312*)

Table 2 (*continued*)

Matrix	Pesticide	Recovery (%)	Concentration range ($\mu g\ l^{-1}$)	LD (mg kg^{-1})	MRLs (mg kg^{-1})	Reference
Cherries, Grapes	Diazinon	91–103	0.05–5.0	0.004	0.5	
Melons, Pepper	Dichlorvos	84–92	0.01–0.5	0.005	0.1	
Plums, Potatoes	Dimethoate	89–106	0.02–1.0	0.003	1.0	
Raspberries	Ditalimphos	82–92	0.05–1.0	0.003	–	
Tomatoes	Etrimfos	82–96	0.05–5.0	0.008	0.05–0.5	
Apples, Apricots	Fenitrothion	91–103	0.05–1.0	0.005	0.5–2.0	[101]
Bananas,	Fenthion	87–97	0.05–5.0	0.004	0.05–0.5	
Broccoli	Fonophos	86–94	0.05–1.0	0.004	0.1	
Cucumbers	Formothion	86–98	0.01–0.5	0.004	0.1–0.2	
Currants	Heptenophos	78–102	0.1–1.0	0.01	0.02–0.1	
Eggplant	Malathion	87–94	0.05–2.0	0.01	0.1–3.0	
Lemons,	Methamidophos	82–93	0.05–1.0	0.01	0.01–0.2	
Oranges	Methidathion	87–93	0.05–1.0	0.004	0.02–2.0	
Pears, Radish	Mevinphos	92–99	0.05–1.0	0.002	0.01–0.5	
Beets	Monocrotophos	79–87	0.1–1.0	0.01	0.02	
Brussels sprouts	Parathion-ethyl	96–103	0.05–1.0	0.005	0.2	
Carrots, Celery	Parathion-methyl	90–102	0.02–1.0	0.003	0.2	
Green beans	Phenthoate	78–94	0.02–2.0	0.004	0.05–1.0	
Green peas	Phorate	87–98	0.01–0.5	0.005	0.01	
Kohlrabi	Phosalone	73–86	0.05–1.0	0.01	0.1–2.0	
Lettuce	Phosmet	82–93	0.1–2.0	0.01	0.05–5.0	
Turnip	Phosphamidon	80–96	0.05–1.0	0.01	0.15–2.0	
	Pirimiphos-methyl	87–94	0.05–0.5	0.004	0.2–2.0	
	Prothoate	81–94	0.05–1.0	0.01	–	
	Pyrazophos	82–101	0.1–1.0	0.004	0.01–0.2	
	Quinalphos	90–108	0.05–1.0	0.01	0.01–0.3	
	Terbufos	87–93	0.05–1.0	0.01	0.05	
	Tetrachlorvinphos	82–98	0.05–1.0	0.01	0.05–0.5	
	Tetradifon	87–103	0.05–1.0	0.005	0.02–3.0	
	Thiometon	91–98	0.05–1.0	0.01	0.03–0.5	
	Phenyl-ureas (Herbicides)					
	Chlorbromuron	79–87	0.1–1.0	0.1	–	[101]
	Linuron	69–78	0.2–0.5	0.08	0.05–3.0	
	Metobromuron	68–81	0.05–2.0	0.08	0.02	
	Pyrethrines (Insecticides)					
	Alphametryne	82–93	0.2–0.5	0.005	–	[101]
	λ-cyhalotrine	75–87	0.1–1.0	0.002	0.01–0.5	
	Cyhalothrin	92–102	0.1–0.5	0.002	0.01–0.5	[102]
	Cypermethrin	76–213	0.05–1.0	0.002	0.05–2.0	[101,102]
	Eltamethrin	77–137	0.05–0.5	0.001	0.05	
	Fenpropathrin	77–106	0.1–1.0	0.05	0.02–2.0	
	Fenvalerate	82–101	0.1–1.0	0.002	0.05–2.0	
	Fluvalinate	82–96	0.05–1.0	0.006	0.01–1.0	[101]
	Permethrin	80–103	0.1–1.0	0.01	0.05–1.0	[101,102]

Table 2 (*continued*)

Matrix	Pesticide	Recovery (%)	Concentration range ($\mu g\ l^{-1}$)	LD ($mg\ kg^{-1}$)	MRLs ($mg\ kg^{-1}$)	Reference
Cherries, Grapes	*Acaricides*					
Melons, Pepper	Bromopropylate	90–95	0.05–1.0	0.01	1.0–3.0	[101]
Plums, Potatoes	Chloropropylate	83–95	0.05–1.0	0.008	–	
Raspberries	Dienochlor	87–101	0.01–0.2	0.0006	–	
Tomatoes	DNOC	69–79	0.01–0.5	0.001	–	
Apples, Apricots	Flubenzimine	65–78	0.05–1.0	0.05	0.01–1.0	
Bananas,						
Brocolli	*Other herbicides*					
Cucumbers	Alachlor	80–90	0.1–0.5	0.02	0.05	[101]
Currants	Benefin	90–100	0.01–0.5	0.001	–	
Eggplant	Butylate	80–96	0.05–0.5	0.01	0.03–0.05	
Lemons	Dinosebacetate	81–96	0.05–1.0	0.005	0.05	
Oranges	Diphenamid	76–82	0.05–1.0	0.01	0.05	
Pears, Radish	Ioxynil	79–83	0.1–1.0	0.003	–	
Beets	Fluchloridone	78–93	0.005–1.0	0.1	–	
Brussels sprouts	Nitrofen	69–82	0.5–2.0	0.01	0.05	
Carrots, Celery	Oxyfluorfen	82–94	0.05–1.0	0.01	0.05	
Green beans	Pendimethalin	81–93	0.05–1.0	0.004	0.05	
Green peas	Phenkapton	86–93	0.05–2.0	0.01	–	
Kohlrabi	Propachlor	92–102	0.05–1.0	0.05	0.05	
Lettuce	Terbacil	78–90	0.1–1.0	0.02	0.05	
Turnip	Trifluoroalin	91–100	0.05–1.0	0.005		
	Fungicides					
	Bitertanol	85–95	0.1–0.5	0.1	0.05	[101]
	Bupirimate	84–94	0.1–2.0	0.02	0.05–0.5	
	Captafol	78-85	0.05–0.5	0.01	0.02	
	Captan	83–93	0.05–0.5	0.005	0.5	
	Chlorothalonil	83–97	0.1–1.0	0.01	0.01	
	Dichlobutrazol	73–89	0.05–0.5	0.005	0.05	[101]
	Dichlozoline	75–87	0.05–0.5	0.02	–	
	Dimethrimol	82–93	0.01–0.5	0.02	0.1	
	Etaconazole	78–89	0.05–0.5	0.04	–	
	Ethirimol	83–92	0.05–1.0	0.1	0.05–0.5	
	Fenarimol	78–93	0.01–0.5	0.01	0.02–0.2	
	Folpet	87–95	0.01–1.0	0.005	0.05	
	Iprodione	82–96	0.1–2.0	0.01	0.02–10.0	
	Metalaxil	76–89	0.1–1.0	0.01	0.05–0.2	
	Nuarimol	87–94	0.05–0.5	0.01	0.01–0.2	
	Procymidone	78–89	0.1–2.0	0.01	0.05–0.2	
	Propioconazole	78–89	0.1–2.0	0.01	0.05–0.2	
	Traidimefon	87–98	0.1–1.0	0.03	0.05–1.0	
	Vincozolin	91–99	0.05–1.0	0.005	0.05–5.0	

(*Continued on p. 314*)

Table 2 (*continued*)

Matrix	Pesticide	Recovery (%)	Concentration range ($\mu g\ l^{-1}$)	LD ($mg\ kg^{-1}$)	MRLs ($mg\ kg^{-1}$)	Reference
Cherries, Grapes	*Other structures*					
Melons, Pepper	Acetochlor	85–96	0.05–0.5	0.01	–	[101]
Plums, Potatoes	Aziprotryn	90–98	0.1–2.0	0.02	0.05–0.5	
Raspberries	Benalaxil	73–87	0.2–1.0	0.01	0.01	
Tomatoes	Benzopropyl-ethyl	93–105	0.1–0.5	0.01	–	
Apples, Apricots	Ciobutyd	78–92	0.1–1.0	0.08	–	
Bananas,	Cyprofuran	73–84	0.01–0.1	0.05	0.1	
Brocoli	Dimethipin	91–98	0.01–0.5	0.01	–	
Cucumbers	Diathianon	87–96	0.05–1.0	0.01	0.05	
Currants	DNOC	69–79	0.01–0.5	0.001	–	
Eggplant	Fenpropiomorph	79–85	0.1–1.0	0.1	0.2–0.5	
Lemons,	Fluazifop-butyl	69–81	0.05–1.0	0.01	–	
Oranges	Fosmetilan	80–94	0.1–1.0	0.01	0.02	
Pears, Radish	Haloxyfop	78–89	0.05–0.5	0.04	0.05–1.0	
Beets	Hexythiazox	76–83	0.1–2.0	0.01	–	
Brussels sprouts	Mercaptodimetur	81–97	0.05–2.0	0.08	–	
Carrots, Celery	Nitrothaleisoprop	76–89	0.05–1.5	0.01	–	
Green beans	Oxazolin	72–87	0.1–1.0	0.02		
Green peas						
Kohlrabi						
Lettuce						
Turnip						

etables, followed by GC–MS [113]. Recoveries obtained were over 80%, except for methamidophos, which was not recovered at all. This compound was extracted by an SFE method, which allows recoveries of over 70% to be obtained from pepper, cucumber and tomato samples [114].

Comparison of the employment of SFE–CO_2 with and without methanol as modifier to extract bound ^{14}C residues from onion and radish has been described [111]. In this paper, ^{14}C material extracted was trapped in methanol, radioassayed, and analyzed by GC with NPD and ECD. Results demonstrated that SFE–CO_2 modified with methanol improved the recovery of bound ^{14}C residues.

Thiocarbamate pesticide residues obtained from apples by SFE and GC–FID, HPLC with sulfur chemiluminiscence detector (SCD) or HPLC–UV for extract analysis, were compared [107]. A tandem trapping technique, which consists first of a solid-phase trap followed by a liquid trap, to avoid problems with analyte trapping when CO_2 modified by methanol and solid-phase trapping is combined, was developed. Results obtained with a simple one-step extraction by SFE are comparable with these obtained by liquid solvent extraction.

SFE was evaluated for the extraction of carbendazime residues in lettuce samples [109] and benzimidazole fungicides in potatoes, apples, and bananas [112]. In the first case, HPLC with fluorescence detection was employed, and in the second one, detection was carried out with an ultraviolet detector.

Analytical SFE is currently a developing technique in which many experimental parameters and problems have yet to be properly defined. The influence of some parameters, such as the pressure and the temperature of the extraction fluid, are now well mastered; others (extraction cell configuration, fluid flow-rate through the extraction cell, period of extraction, sample matrix effects, etc.) need further studies. Similarly, the sample size needs to be optimized.

4. Instrumental analysis

4.1. Gas chromatography (GC)

In recent years, capillary columns have almost completely replaced the packed columns owing to their high resolving power, which allows the sepa-

Table 3
Determination of pesticide residues in fruits and vegetables by MSPD with C_{18}

Matrix	Pesticide	Concentration range ($\mu g\ l^{-1}$)	Recoveries (%)	LD ($mg\ kg^{-1}$)	LMRs ($mg\ kg^{-1}$)	Reference
	Carbamates					
Apple	Oxamyl	20–1000	84–129	0.02	0.05–3.0	[103]
Orange	Methomyl	20–1000	80–120	0.02	0.02	
Grapefruit						
Lemon	*OCPS (Insecticides)*					
Pear	Aldrin	19	35–101	0.02	–	[104]
Plum	Dicofol	65–1000	55–106	0.01–0.012	0.02	[104,105]
Lettuce	α-Endosulfan	50–1000	56–103	0.015	1.0	
Tomato	β-Endosulfan	36–1000	70–101	0.015	1.0	
	Fungicides					
	Captafol	625–1000	24–87	0.017–0.171	0.5–2.0	
	Folpet	51–1000	63–99	0.009–0.012	0.5–3.0	[104,105]
	OPPs (Insecticides)					
	Azinphos-methyl	498	57–95	0.008	0.01	
	Carbophenothion	96–1000	64–94	0.018–0.024	0.02–2.0	
	Chlorfenvinphos	179–1000	58–99	0.020–0.028	0.05–1.0	[104]
	Chlorpyriphos	19–1000	70–94	0.010–0.5	0.2–0.5	[104,105]
	Diazinon	976–1000	58–79	0.056–0.1	0.5	
	Ethion	539–1000	64–91	0.004–0.5	0.1–2.0	
	Fenitrothion	19–1000	65–108	0.012–0.5	0.5–3.0	
	Malathion	160–1000	60–92	0.024–0.5	0.02–2.0	
	Methidathion	179–1000	45–88	0.024–0.5	0.2	
	Parathion-methyl	115–1000	66–105	0.018–0.5	0.5–5.0	
	Phosmet	230–1000	59–88	0.03–0.5	1.0–2.0	
	Tetradifon	65–1000	55–108	0.017		

ration of a large number of pesticides with similar physico-chemical characteristics [25].

As has been widely discussed, extracts of many commodities include indigenous compounds that can interfere with chromatography, so most modern methods employ selective detectors. An ideal selective detector for residue analysis would respond only to the target pesticides, while other coextracted compounds remain transparent [25]. Table 5 summarizes the different detectors employed for the determination of different types of pesticides in fruit and vegetables.

The most frequently used detectors include ECD, NPD, FPD and MSD. This last one has become the standard confirmatory technique. MIP–AED, which allows the specific detection of many elements, has recently been applied to the determination of pesticides.

In the past 30 years, the ECD has been the detector most used in pesticide residue analysis. It presents a very high sensitivity to polychlorinated hydrocarbons and other halogenated pesticides but its selectively is rather poor [72] because all kinds of electron- attracting functional groups such as nitro groups and aromatic structures also give a reponse on this detector [117,118]. OCPs, OPPs and pyrethroid pesticides had been determined with this detector from several matrices [26,31,59,63,65,69,76,84,85,128]. Therefore, the interpretation of ECD chromatograms obtained from extracts with higher amounts of matrix compounds (as occurs with those obtained from leek, garlic, onion, cabbage and others) can become a difficult task. Two-dimensional capillary GC using the techniques of heart-cutting and backflush makes it possible to transfer small fractions or even single peaks to a second column, where all relevant pesticides can be separated from their overlapping matrix compounds [117]. However, ECD is normally used together with other element selective detectors in

Table 4
Supercritical fluid extraction of pesticides from fruit and vegetables

Matrix	Pesticide	Concentration range ($\mu g\ l^{-1}$)	Recoveries range (%)	LD ($mg\ kg^{-1}$)	LMRs ($mg\ kg^{-1}$)	Reference
	Benzimipazole (Fungicides)					
Apple	Carbendazim	300–12000	54–98	0.05–0.1	0.1–5.0	[109,112]
Banana	Thiabendazole	25–1000	72–88	0.0012	0.1–6.0	[112]
Lettuce						
Potato						
	Carbamate (Insecticides)					
Apple	Carbaryl	500	91	0.005	1.0–5.0	[113]
Broccoli	Carbofuran	500	90	0.002	0.1–2.0	
Carrot	Chlorpropham	500	91	0.005	0.05–0.1	
Grape	Eptam	500	31	0.005	–	
Potato	Methiocarb	500	74	1.005	0.05–1.0	
	Methomyl	500	84	0.005	0.02–1.0	
	OCPS (Insecticides)					
Broccoli	Chlorothalonil	500	93	0.002	0.01–2.0	[113]
Carrot	Dacthal	500	85	0.0004	–	
Grape	DDE	500	91	0.0017	0.05	
Onion	DDT	500	93	0.001	0.05	
Potato	Dieldrin	500	89–92	–	0.01	
Radish	Endosulfan I	500	93	0.007	1.0	
	Endosulfan II	500	114	0.008	1.0	
	Fonofos	670	92–94	–	0.1	
	Hexachlorobenzene	500	93	0.002	0.01	
	Lindane	500	89	0.004	0.1–1.0	[111]
	Methoxychlor	500	90	0.003	10.0	[113]
	Pentachlorobenzene	500	91	0.002	–	[111]
	Pentachloronitrobenzene	500	90	0.003	–	[113]
	OPPS (Insecticides)					
Broccoli	Azinphos-methyl	500	94	0.15	0.5–2.0	[113]
Carrot	Chlorpyrifos	500	72	0.02	0.05–3.0	
Grape	Diazinon	500	86	0.002	0.5	
Onion	Dichlorvos	500	72	0.006	0.1	
Potato	Dimethoate	500	83	0.004	1.0	
Radish	Disulfoton	500	78	0.004	0.02	
	Ethion	500	97	0.006	0.1–2.0	
	Ethoprop	500	84	0.006	0.02	
	Fenamiphos	500	83	0.005	0.02–0.2	
	Malathion	500	87	0.006	0.1–3.0	
	Methamidophos	500	–	0.014	0.01–0.2	
	Methidathion	500	90	0.009	0.02–2.0	
	Mevinphos	500	92	0.002	0.01–0.5	
	Omethoate	500	5	0.02	0.1–0.4	
	Parathion	500	91	0.018	0.2	
	Parathion-methyl	500	85	0.006	0.2	
	Phorate	500	82	0.002	0.01	
	Phosalone	500	86	0.017	0.1–2.0	
	Phosmet	500	88	0.042	0.1–2.0	
	Phosphamidon	500	91	0.027	0.15–2.0	
	Terbufos	500	83	0.003	0.05	

Table 4 (*continued*)

Matrix	Pesticide	Concentration range ($\mu g\ l^{-1}$)	Recoveries Range (%)	LD ($mg\ kg^{-1}$)	LMRs ($mg\ kg^{-1}$)	Reference
	Pentachloro-nitrobenzenes					
Carrot	PCB					
Celery	TCNB	100	84–100	0.028	–	[110]
Green beans	HCB	100	78–127	0.021	–	
Potato	PCAS	200	78–99	0.014	–	
Radish	PCNB	100	71–101	0.01	–	
	PCAL	100	82–112	0.024	–	
	PCTA	100	78–98	0.019	–	
		100	68–106	–	–	
	Pyrethroids					
Broccoli	Esfenvalerate	500	88	0.013	0.05–2.0	[113]
Carrot	Fenvalerate	500	93	0.029	0.05–2.0	
Grape	*cis*-Permethrin	500	93	0.013	0.05–1.0	
Potato						
	Other structures					
Brocolli	Atrazine	500	92	0.004	0.05–0.2	[113]
Carrot	Captan	500	66	0.01	0.5	
Cucumber	Dicloran	500	91	0.018	–	
Grape	Diphenylamine	500	87	0.003	0.05–3.0	
Pepper	Iprodione	500	102	0.005	0.02–10.0	
Potato	Methamidophos	50–250	43–78		0.01–0.2	
Tomato	Myclobutanil	500	83	0.048	0.01–0.5	
	Propargite	500	57	0.009	0.05–5.0	
	Vinclozolin	500	91	0.004	0.05–5.0	

monitoring programs [72]. Usually, the extracts obtained are injected into various of the detectors mentioned above because their combined use makes better identification and detection of the pesticide residues possible. The most used combination of detectors is ECD, NPD and FPD

Table 5
Recommended detectors for the determination of different types of selected pesticides

Pesticide Group	ECD	NPD	FPD	ELCD	MIP–AED	MSD	References
OCPs	xxxxxxxxxxx xxxxxx	x		xxx	xxx	xxxxx	[33,38–40,54,57,59,64,66,70–73, 101,104,105,115–120]
OPPs	xxxxxxxxx	xxxxxxxxx xxxxxxxx	xxxxxxxx xxxxxxxx xxxxx	xx	xxx xxxxx	xxxxxx	[33,38–44,47,50,52–56,60,62,64, 66–68,70–73,77,83,101,104,105, 112,115–124]
Chlorotriazines	x	xx			xx		[38,82,101,118,125]
Carbamate		xxxxxxx	x		xx	xxx	[40,43,49,56,66,73,101,118–120,122]
Pyretrhroide	xxxxxxxxxx				xxxxx		[45,66,69,83–85,101,102,116,118]
Fungicides	xxxxxxxxxxx	xxxxx	x			xxx	[31,39,40,45,46,57,58,61,63,64, 66,83,101,118,126,127]
Chlorinated herbizides	xxxxxxx	x					[38,57,65,87,88,96,101]
Dithiocarbamates		x	x				[49,81]
Glyphosate					x		[89]
Quats		x			x		[96]

x: number of times used in the literature

[33,35,40,66,72,117]. Other combinations employed are those formed by ECD, FPD and electrolytic conductivity detector (ELCD) [45,54,115], or ECD, NPD, FPD, ELCD and AED [72,120]. The detectors are sometimes connected in parallel, since it allows the results to be obtained with only one injection [33,72,115].

NPD is an important detector used in pesticide residue analysis, because of its selectivity for phosphorus and nitrogen containing compounds. The sensitivity of this detector is usually better for phosphorus than for nitrogen. OPPs, carbamates, triazines and their metabolites, and fungicides were determined by NPD from different fruit and vegetables [47,55,82,122,126].

The FPD, in phosphorus mode, has frequently been the instrumental technique of choice for the analysis of OPPs [41,42,44,60,62,67,68,77]. Interferences in analytical determinations may occur when FPD-P is used in the phosphorus mode and sulphur is present [38,41], and when some thermally labile organophosphate pesticides, such as trichlorfon, are present or when blending of the liquid phase occurs [41]. Dithiocarbamate insecticides can be also determined with this detector as CS_2 [81].

A new detector introduced in 1989, the AED, is used for its selective detection of the elements fluorine, chlorine, bromine, iodine, phosphorus, sulphur and nitrogen. Its applicability was compared with other element selective detectors and showed higher selectivity in the determination of chlorine-, fluorine- and phosphorus-containing pesticides than other detection methods for 12 agricultural products [120]. Carbamate, pyrethroid, organochlorine and organophosphorus pesticides was also determined by GC–MIP-AED from fruit and vegetables [72,119].

MSD can be employed to achieve selective detection, by full scan or selective ion monitoring, of target pesticides in the presence of the complex matrix. MSD is a highly sensitive and specific technique suitable for use in environmental organic analysis. The most widely used technique for pesticide residues analysis is MSD with electron impact (EI) ionization. Quantification is usually achieved by the technique of selected ion monitoring (SIM). With this technique selectivity is also improved [33,41,44,45,72,89,118,125,127,129].

Its selectivity can be enhanced by either the use of different reagent gases in the positive chemical ionization (PCI) and/or negative chemical ionization (NCI) modes or by the use of two or more techniques in tandem, such as GC–MS–MS. The presence of OPPs [123] and 20 suspected oncongenic pesticides [116] in crop samples were confirmed by CI–MSD. Carbamate pesticides were also confirmed by MDS in positive ion chemical ionization (PICI) mode [130]. A multiresidue method for screening OPP residues in fruit and vegetable samples, with an ion trap mass spectrometer in the chemical ionization mode, was developed [121,124].

4.2. High-performance liquid chromatography (HPLC)

Nowadays, HPLC is being extensively used in pesticide chemistry and related areas where the chemicals of interest are frequently of low volatility (bipyridylium herbicides) or thermally unstable (benzoylurea or *N*-methylcarbamates) for GC separation. The diverse methods used to determine pesticides in food samples by HPLC have been well documented by Bushway [131]. Because of its importance, some recently published methods of analyses for pesticide residues in fruit and vegetables are shown in Table 6.

Summarizing them, HPLC methods for the determination of pesticide residues in fruits and vegetables could employ reversed-phase chromatography with C_{18} or C_8 columns and aqueous mobile phase, followed by UV absorption [138,142], UV diode array [34], mass spectrometric [143,150] or fluorescence [135,136,151] detection.

In the determination of bipyridylium herbicides by HPLC, which are ionic compounds, an ion-pairing reagent in the mobile phase [90–92] is used to achieve an effective separation of them. The simultaneous determination of Diquat and Paraquat, using a UV detector [90–92] or diode-array UV detector [90], has been reported. Mepiquat chloride has been determined in animal and plant matrices by ion chromatography with conductivity detection [97].

N-methyl carbamates can be determined using a UV detector [32,132]. However, the sensitivity and selectivity offered by UV detection is very poor, because the carbamates present their absorption maximum about 190 nm. They do not posses native

Table 6
Methods of analyses for pesticide residues in fruit and vegetables

Pesticide	Matrix	HPLC Column	Eluent	Detection	Reference
Butocarboxim Oxamyl Methomyl Meth. sulphoxide Aldicarb Butocarboxim Carbofuran Propoxur Bendiocarb Carbaryl Thiofanox Ethiofencarb 1-Naphtol Isoprocarb Landrin	Apple Carrot Cauliflower Celery Cucumber Leek Onion Orange Potato Spinach Strawberry	LiChroCart packed with LiChrosphere 100 RP-18	$ACN-H_2O$ isocratic	Fluorescence λ_{ex} 340 nm λ_{em} 455 nm	[79]
MK-0244 Delta-8,9-isomer	Celery Lettuce	RP-18 OD-GU	$MeOH-H_2O$ isocratic	Fluorescence λ_{ex} 340 nm λ_{em} 455 nm	[137]
Carbendazim Thiabendazole	Pear Apple Orange Grape Kiwifruit	Ashipak ODP-50 filled with Spherisorb RP-18	$ACN-H_2O$ isocratic	UV 280 nm Fluorescence λ_{ex} 280 nm λ_{em} 310 nm	[86]
Iprodione Vinclozoline Procimidone	Carrot Fennel Onion	Hypersil ODS	$ACN-H_2O$ gradient	UV 229 nm	[138]
Mepiquat	Onion Garlic	Ion pack column MPIC-NG1	Hexane–sulphonic acid with 3% ACN isocratic	Conductivity	[97]
Butocarboxin sulfoxide Aldicarb sulfoxide Butoxycarboxim Aldicarb sulfone Oxamyl Methomyl Ethiofencarb sulfoxide Thiofanox sulfoxide 3-Hydroxycarbofuran Ethiofencarb sulfone Methiocarb sulfoxide Dioxacarb Thiofanox sulfone Methiocarb sulfone Aldicarb 3-Ketocarbofuran Propoxur Carbofuran Carbaryl Ethiofencarb Isoprocarb Carbanolate Methiocarb Promecarb Bufencarb	Apple Banana Carrot Cauliflower Endive Onion Orange Paprika Peach Potato Strawberry	LiChroCART 4×4 mm id	$ACN-H_2O$ gradient	Fluorescence λ_{ex} 340 nm λ_{em} 455 nm	[80]

(Continued on p. 320)

Table 6 (*continued*)

Pesticide	Matrix	HPLC Column	Eluent	Detection	Reference
Aldicarb sulfoxide Aldicarb sulfone Oxamyl Methomyl 3-Hydroxycarbofuran Aldicarb Propoxur Carbofuran Carbaryl Methiocarb	Apple Broccoli Cabbage Cauliflower Potato	Apex ODS	MeOH–H_2O gradient	Fluorescence λ_{ex} 340 nm λ_{em} 455 nm	[139]
Aldicarb Ethiofencarh Methiocarb	Apple Banana Cabage Carrot Cucumber Egg plant Grape Potato Strawberry	Shimazu STR-ODS II	MeOH–H_2O gradient	Fluorescence λ_{ex} 340 nm λ_{em} 455 nm	[140]
Triazophos Chlorpyriphos Diazinon Pirimiphos-methyl Fenitrothion HCH DDE Dieldrin DDT TDE Tecnazene Dinoseb Chlorpropham Dichlorphen Carbendazim Thiabendazole 2-Aminobutane Captafol	Potatoes	Spherisorb ODS(C_{18})	MeOH–H_2O isocratic with 0.6% ammonia solution	UV 216 nm Fluorescence λ_{ex} 296 nm λ_{em} 351 nm	[59]
Aldicarb sulfoxide Oxamyl Methomyl 3-Hydroxycarbofuran Propoxur Carbofuran Carbaryl Methiocarb	Asparagus Broccoli Cabbage Carrot Cauliflower Celery Cucumber Eggplant Endive Escarole Lettuce Mushroom Onion Pea Pepper Potato Rhubarb	Zorbax C-18, and Zorbax CN, spherical particles	ACN–H_2O gradient	Fluorescence λ_{ex} 340 nm λ_{em} 455 nm	[78]

Table 6 (*continued*)

Pesticide	Matrix	HPLC Column	Eluent	Detection	Reference
	Spinach				
	Squash				
	Tomato Zucchini				
	Apricot				
	Banana				
	Grape				
	Grapefruit				
	Lemon				
	Lime				
	Peach				
	Pear				
	Prune				
	Orange				
	Strawberry				
Ethylenethiourea	Tomato	Nucleosil C_{18}	MeOH–H_2O	Amperometric	[141]
	Cucumber		isocratic	1500 mV	
Diflubenzuron	Apples	Separon SGX C_{18}	MeOH–H_2O	Diode-array	[142]
Triflumuron		Separon SGX Phenyl	AcN–H_2O		
Flufenoxuron		Separon SGX C_8			
Chlorfluazuron					
Flucycloxuron					
Diquat	Asparagus	Du Pont Zorbax silica	NaCl–H_2O–AcN	Diode-array	[90,93]
Paraquat	Potato		pH= 2.2	UV/VIS	
	Turnip			(257–310 nm)	
Aldicarb	Apple	Spheri-5	Amonium acetate–AcN–H_2O	Thermospray–MS	[143]
Aldicarb sulfoxide	Lettuce	reversed-phase C-18	isocratic		
Bufencarb	Pepper				
Carboxim	Potato				
Chlorbromuron	Tomato				
Diuron					
Linuron					
Methiocarb					
Methomyl					
Metobromuron					
Monuron					
Neburon					
Oxamyl					
Propoxur					
Thiodicarb					
Aldicarb	Tomatoes	RP-18	MeOH–H_2O–THF	Fluorescence	[144]
Bendiocarb	Shallot		gradient	λ_{ex} 339 nm	
Carbaryl	Banana			λ_{em} 440 nm	
Carbofuran	Lettuce				
Ethiofencarb	Cucumber				
Fenobucarb	Carrot				
Isoprococarb	Pimento				
Methiocarb	Japanish Pear				
Metomil	Cherry				
Metolcarb	Kiwifruit				
Oxamyl	Lemon				
Proporxur	Mitsuba				
Thiodicarb					
XM-C					
MP-MC					

(*Continued on p. 322*)

Table 6 (*continued*)

Pesticide	Matrix	HPLC Column	Eluent	Detection	Reference
Curaterr	Asparagus	Spherisorb RP-C18	MeOH–H_2O	UV 254 nm	[145]
Carbaryl	Cauliflowers	Lichrosorb	isocratic	Fluorescence	
Cronetron	Hops	SI-100 RP-C18		λ_{ex} 340 m	
Etrofolam	Radish	Homemade RPC-18 base on Grace silica gel		λ_{em} 455 nm	
Tribunyl	Cabbage				
Bassa					
Mesurol					
Ethiofencarb	Lettuce	Hibar RP-18	ACN–H_2O isocratic	UV 190 nm	[146]
Carbofuran	Tomato	C_8 NovaPack	MeOH–H_2O–ACN	Fluorescence	[147]
Aldicarb			gradient	λ_{ex} 345 nm	
Aldicarb sulphone				λ_{em} 455 nm	
Bendiocarb					
Carbaryl					
Carbofuran					
Methiocarb					
Methomyl					
Mexacarbate					
Oxamyl					
Proporxur					
Aldrin					
BHC					
Chlordane					
DDE					
DDD					
DDT					
Dicofol					
Dieldrin					
Endosulfan					
Endrin					
HCB					
Heptachlor					
Heptachlorepoxide					
Lindane					
Metoxychlor					
Mirex					
Acephate					
Azinphos-methyl					
Carbofenotion					
Chlorfenvinfos					
Chropyrifos					
Cumafos					
Demeton	Tomato	C_8 Nova Pack	MeOH–H_2O–ACN	Fluorescence	[147]
Diazinon			gradient	λ_{ex} 345 nm	
Dicrotophos				λ_{em} 455 nm	
Dimethoate					
Dioxation					
Disulfoton					
EPN					
Ethion					
Etoprop					
Fenamifos					
Fensulfothion					
Fenthion					

Table 6 (*continued*)

Pesticide	Matrix	HPLC Column	Eluent	Detection	Reference
Fonophos					
Isofenphos					
Malathion					
Merphos					
Methamidophos					
Methidathion					
Methyl-parathion					
Mevinphos					
Monocrotophos					
Naled					
Parathion					
Phorate					
Phosalone					
Phosmet					
Phosamidon					
Profenophos					
Propetanphos					
Ronnel					
Terbuphos					
Tetrachlorvinphos					
Triazophos					
Carbofuran	Melon	Lichrocart packed with	ACN–H_2O	UV 254 nm	[148]
Propoxur	Cucumber	Supersphere R8	isocratic		
Bendiocarb	Plum				
Dioxacarb	Apple				
Etiophencarb	Paprika				
Isoprocarb	Strawberry				
Landrin					
Carbaryl					
Carbanolate					
Methiocarb					
Oxamyl					
Methomyl					
Butocarboxim					
Aldicarb					
Thifanox					
Butocarboxim	Apple	Zorbax C_8	ACN–H_2O	Fluorescence	[149]
Aldicarb	Lettuce	Spherisorb RP-18	gradient	λ_{ex} 340 nm	
Butoxicarboxim	Tomato			λ_{em} 455 nm	
Oxamyl	Sugar Beets				
Ethiofencarb					
Methomil					
Methiocarb					
Carbofuran					
Dioxacarb					
Butocarboxim					
Bendiocarb					
Carbaryl					
Thiofanox					
Trimethacarb					
Etrofolan					
Methiocarb					
Baycarb					
Promecarb					

fluorescence either, but they can be made to fluoresce by derivatization [152].

Quantification of carbamates by HPLC using postcolumn derivatization and fluorescence detection was first described by Moye et al. [153]. This technique was modified for use on food samples by Krause [20], and nowadays it is the favourite technique for analysts [139,140,144–149]. In recent years de Kok et al. reported an improved procedure using SPE clean-up and automated injection [79,80]. Page and French use this procedure in conjunction with the Luke's method extraction [78].

A method reported recently for the analysis of ethylene bisdithiocarbamate (ETU) consists of HPLC with amperometric detection [141]. The method was applied to the determination of trace amounts of ETU in tomatoes and cucumbers. The minimum quantitation level was 0.01 mg kg^{-1}.

A newly discovered family of pesticidal agents are the macrocyclic lactones produced by the actinomycetes. The purified extracts of vegetables were analyzed by HPLC with UV [133,134] or fluorescence [137] to detect this compounds. The detection limit of the methods are about 2 μg kg^{-1}.

4.3. Supercritical fluid chromatography (SFC)

SFC is a chromatographic technique that in many ways, is a hybrid of GC and HPLC. It is recognized as a valuable technique for the analysis of thermolabile compounds which would not be amenable to analysis by of GC or HPLC. Few applications have been reported for SFC in the field of pesticide determination from fruit and vegetables [154–157]. They are presented in Table 7.

Advantages of the SFC are the versatility in separation (by addition of modifier, choice of stationary phase) and detection (by use of LC or GC detectors). It also offers the possibility of direct coupling of SFE–SFC [108] that makes possible the selective extraction of analytes with a small amount of organic solvent and their introduction into the

Table 7
Supercritical fluid chromatography of pesticide residues in fruit and vegtables

Pesticide	Matrix	SFC Conditions	Detection	Reference
Triazine and Triazole Herbicides	Cherry	CO_2–MeOH gradient elution	UV	[155]
Esfenvalerate Diniconazole Fenitrothion	Cucumber		UV	[108]
Azinphos-methyl Dimethoate Ethione Malathion Phoxim	Onion Tomato	CO_2–MeOH (1.5–5.7%) 36–50 °C 78–91 bar CO_2–2-propanol (3.5%) 47 °C–84 bar	NPD	[156]
Azinphos-methyl Carbophenothion Diazinon Dichlorvos Dimethoate Disulfoton Ethione Malathion Paraoxon Parathion-ethyl Phorate	Cucumber Grape Lettuce	CO_2–MeOH (1.5–5.7%) 36–50 °C 78–91 bar CO_2–2-propanol (3.5%) 47 °C–84 bar	NPD	[157]

chromatograph without injection. However, SFC is a little used technique because it still presents instrumental problems.

4.4. Immunoassay (IA)

IA provides rapid, sensitive, and cost effective analyses for a variety of pesticide residues. However, fast progress in the analytical determination of pesticides with either HPLC or GC separation and selective detection, clearly demonstrates that IA cannot compete in terms of the information obtained about the sample composition. The main disadvantage is that only one compound at a time can be determined. The usefulness of these techniques is experienced during screening analyses when a large number of samples have to be analyzed in parallel for a single analyte within a short time. They supplement traditional analytical methods, because of their extreme sensitivity, simplicity and low cost [158]. A recent review [159] showed that the most important types presently used for pesticide analysis are immunoassays (IAS), immunosensors (IS), immunoaffinity chromatography (IAC), and immunolabeling (IL). Those immunoassay techniques, and their application to the detection of pesticide and drug residues in food, had been reported recently by Bushway et al. [160]. Table 8 summarizes the results reported of their application to fruits and vegetables.

Among the different IA procedures the most explored format was enzyme-linked immunoabsorbent assay (ELISA). Traditionally ELISA was applied to the analysis of water samples, but it has been extended to the analysis of more complex matrices such as fruit juices [148,160,169–171]. Nowadays, there are already several studies which indicate that ELISA can be used to analyze agricultural products after solvent extraction [161–167,172–174]. However, an erroneous result could be produced by matrix effects or the inability to differentiate between structurally similar compounds (cross reactivity).

Further progress with the IA technique led to the development of IS. These are devices which use immobilized biomolecules in an electrode to detect chemicals through their specific interactions. They are commonly considered as biosensors although the future use of recombinant antibodies (Abs) or other synthetic binding proteins may not totally justify this classification [159]. Biosensors for the detection of pesticide residues are based on the inhibition of acetylcholinesterase (AChE) or cholinesterase (ChE), combined with a variety of transducers. For the assay of organophosphorus and carbamates, the cholinesterase enzymes can be considered as the key enzymes [175,176]. These methods have been applied to different fruits and vegetables [168].

Up to now IAC or IL have not been reported for pesticide residue determination in fruits and vegetables [159].

4.5. Other techniques

Different techniques have also been proposed to determine pesticide residue contents in fruit and vegetables. A simplified indirect method for the determination of the total content of polychlorinated organic compounds in waters, soils and plants was developed using adapted versions of molecular emission cavity analysis based on measurements of the intensity of the emission band of indium monochloride at 359.9 nm [177]. The detection limit was 0.05 ng of chlorine. The proposed technique is suitable for the evaluation of the contamination level of plants with polychlorinated organic pesticides.

Polarographic methods for the determination of the organochlorine pesticides dieldrin, heptachlor, endosulfan and endosulfan sulphate in emulsions formed by ethyl acetate and a mixture of two surfactants, Hyamine 2389 and Triton, have been reported recently [178,179]. Polarography in an oil–water emulsified medium is particularly interesting from a practical point of view when the analyte can be extracted from the sample into organic solvents. These emulsified media are predominantly aqueous and minimized the main problems caused by the use of organic solvents in electroanalysis.

A spectrophotometric method for the determination of sub ppm levels of the organophosphorus pesticide ethion based on the oxidation of it by potassium permanganate in phosphoric acid to sulphone, and its hydrolysis under acidic conditions to release formaldehyde, which is determined by a reaction with 1,3,5-trihydroxybenzene in alkaline medium, is applied to different samples of fruits [180].

An analytical procedure for determination of

Table 8
Immunochemical techniques applied to pesticide analysis in fruit and vegetable samples

Pesticide	Matrix	Extraction	Clean-up	Technique	LD (mg kg $^{-1}$)	Possible cross reactions	Reference
Thiabendazole	Apple Potato	Blended in water, centrifuge	–	ELISA	<0.2	–	[161]
Methyl-2 benzimidazole carbamate	Blueberries	Methanol	Alumina	ELISA	0.18	Benomyl Thiabendazole MBC Thiophanate-methyl Thiophanate Procymidone Vinchlozolin	[162]
Paraquat	Potato	HCl	–	ELISA	0.01–0.02	–	[163]
Linuron	Carrot Potato		Silica		0.11–0.61	–	[164]
Chlorothalonil	Celery Snow pea pods	Acetone Methanol	–	ELISA	0.2 0.05	2,4,5,6-Tetrachloro-3-cianobenzamide 2,5,6-Trichloro-4-hydroxyisophtalonitrile 3-Carbamyl-2,4,5-trichlorobenzoic acid Pentachloronitrobenzene Hexachlorobenzene Pentachlorophenol 2,4,5,6-Tetrachlorophenol	[165]

Paraquat	Apple Cabbage Potato Zucchini	Sulfuric acid	–	ELISA	0.1	Paraquat cation Methylbipyridyl methyl sulfonium salt Diethylparaquat Monoquat Morphamquat Diquat Chlormequat Difenzoquat Allyltrimethyl-amonium bromide 4,4-bipyridyl 1-Methyl-4-carboxypyridium Methylamine hydrochloride Picolinic acid (2-Bromoethyl)tri-methylammonium salt	[166]
Procymidone	Pepper	Ethyl acetate–sodium sulphate	Silica	ELISA	0.0006	Procymidone Vinclozolin Iprodione Carbendazime Clozolinate Benomyl Thiabendazole	[167]
Aldicarb Paraoxon	Cabbage Tomato Melon Salad	–	–	Chemiluniniscence	0.04–0.008	–	[168]

thiabendazole residues in pears is described by Capitan et al. [181]. This method involves extracting the chemical from chopped fruit with buffer solution, use of Sephadex G-15 dextrane type gel as a solid support, and determination of TBZ by solid phase spectrofluorimetry (SPF). The relative fluorescence intensity of the Sephadex G-15 gel-TBZ system, packed in a 1 mm thickness silica cell, was measured directly with a solid phase attachment. Identification of the *o*-Phenylphenol, Imazalil, and Thiabendazole residues in citrus fruits by thin-layer chromatography (TLC) has been described [182], and TLC–densitometry suggested as method for the quantitative determination.

The oxidative voltammetric behaviour of the herbicides tetramethyl and tetraethylthiuramdisulfide is reported in order to check the suitability of graphite-PTFE composite electrodes as voltammetric electrodes [183]. The determination of thiran in spiked strawberries was carried out with good results.

A photokinetic method is reported for the determination of diquat in potatoes [95]. A spectrophotometric method [94] is applied successfully to the determination of paraquat in water, grain and plant materials. Finally, the determination of paraquat by flow-injection spectrophotometry [184] or diquat by flow-injection spectrofluorimetry [185] is also described.

5. Conclusions

It is clear that a large and diverse literature exits describing the isolation of pesticides by solvent extraction. This represents a well developed field of study, but one in which there is still much room for further work. They are often lengthy, involve multiple steps and use large volumes of solvent. Solvent disposal is becoming increasingly expensive and environmentally unsound. Therefore, methods using low solvent volumes are desirable.

The newer MSPD techniques offer alternative isolation strategies. When compared to the classical methods, they greatly reduce labor and solvent costs and improve throughput. Although there is tremendous potential shown by MSPD, there are a few drawbacks, and for this reason it needs further development for use with many different types of matrices that may contain residues of chemical contaminants. Only a few references could be found mentioning the use of SFE for fruit and vegetables, mostly because of the effect of the matrix on extraction.

GC and HPLC provide the basis of numerous determination methods alone or in combination with very sensitive and selective detection methods, such as MS. SFC has not gained wide acceptance as an analytical tool, owing to its technical problems.

Immunoassays are beginning to achieve their enormous potential in the field of contaminant residue chemistry. Because immunochemical approaches are based on the attraction between an antibody to an analyte or derivatized analyte, immunochemical techniques can be applied in virtually all stages of trace analysis.

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