RESIDUES AND TRACE ELEMENTS

Evaluation of Two Fast and Easy Methods for Pesticide Residue Analysis in Fatty Food Matrixes

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Two rapid methods of sample preparation and analysis of fatty foods (e.g., milk, eggs, and avocado) were evaluated and compared for 32 pesticide residues representing a wide range of physicochemical properties. One method, dubbed the quick, easy, cheap, effective, rugged, and safe (QuEChERS) method for pesticide residue analysis, entailed extraction of 15 g sample with 15 mL acetonitrile (MeCN) containing 1% acetic acid followed by addition of 6 g anhydrous magnesium sulfate and 1.5 g sodium acetate. After centrifugation, 1 mL of the buffered MeCN extract underwent a cleanup step (in a technique known as dispersive solid-phase extraction) using 50 mg each of C_{18} and primary secondary amine sorbents plus 150 mg $MgSO_4$. The second method incorporated a form of matrix solid-phase dispersion (MSPD), in which 0.5 g sample plus 2 g C_{18} and 2 g anhydrous sodium sulfate was mixed in a mortar and pestle and added above a 2 g Florisil column on a vacuum manifold. Then, 5 2 mL MeCN was used to elute the pesticide analytes from the sample into a collection tube, and the extract was concentrated to 0.5 mL by evaporation. Extracts in both methods were analyzed concurrently by gas chromatography/mass spectrometry and liquid chromatography/tandem mass spectrometry. The recoveries of semi-polar and polar pesticides were typically 100% in both methods (except that basic pesticides, such as thiabendazole and imazalil, were not recovered in the MSPD method), but recovery of nonpolar pesticides decreased as fat content of the sample increased. This trend was more pronounced in the QuEChERS method, in which case the most lipophilic analyte tested, hexachlorobenzene, gave 27 1% recovery (n = 6) in avocado (15% fat) with a <10 ng/g limit of quantitation.

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In the early era of synthetic insecticide development (1940–1960), the main types of insecticides marketed were lipophilic organochlorine (OC) compounds, such as DDT and chlordane, which persist for many years and tend to accumulate in fat. This led to well-documented environmental problems, and the worst of the persistent organic pollutants have been banned in most countries in a series of laws and treaties starting in 1974. Currently, very few commercial sources are available and only limited applications are made of the highly persistent OCs, which has reduced their importance in monitoring programs.

The U.S. Food and Drug Administration (FDA) defines fatty foods as having 2% fat composition, and nonfatty foods have <2% fat (1). However, there is a big difference in the analysis of milk with 3% fat and very fatty samples such as lard. To take this into account, we propose that the terminology should be divided into nonfatty (<2% fat), low fatty (2–20%), and high fatty (>20%) foods, with fat content calculated on a wet weight basis.

Most analytical methods for pesticide residues in fatty foods are designed predominantly for the out-moded OC insecticides and employ solvents such as hexane, acetone, ethyl actetate, and dichloromethane for extraction in order to dissolve the lipids (1–7). However, intensive and time-consuming cleanup, such as gel-permeation chromatography (GPC), is usually needed to remove the coextracted fat from the extracts prior to the analytical step. For high fatty matrixes, such as vegetable oil, animal fat, butter, etc., that consist of >20% lipids, there is no option but to use a nonpolar solvent to dissolve the fat to extract the pesticide residues. In this type of samples, only lipophilic analytes are likely or known to occur (8), so there is little reason to devise methods that must achieve high recoveries of rather polar pesticides in highly lipidic matrixes.

In the case of low fatty matrixes, however, both lipophilic and hydrophilic pesticides can occur (9), and analytical

methods should be devised for those sample types to have a wide polarity range. Many food types have a fat composition of 2–20%, including milk, nuts, wheat, corn, soybeans, other grains, fish, shellfish, other seafood, liver, kidney, meat from poultry, pork, cattle, eggs, and avocado (1, 10). Also, less post-extraction cleanup is needed if the extraction of lipids can be avoided, while still achieving adequate coverage and detection limits for lipophilic analytes. Acetonitrile (MeCN) is a good candidate as an extraction solvent for low fatty matrixes because it gives high recoveries of a wide polarity range of pesticides, and yet it does not significantly dissolve highly nonpolar fats or highly polar proteins, salts, and sugars common in food. Indeed, some methods have been developed for low fatty foods using MeCN for extraction (11–13).

In 2003, Anastassiades et al. (14) introduced the quick, easy, cheap, effective, rugged, and safe (QuEChERS) method for analysis of pesticide residues in fruits and vegetables. In a follow-up study, Lehotay et al. (15) conducted validation experiments of the original sample preparation method for more than 200 pesticides in several matrixes using gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/tandem mass spectrometry (LC/MS/MS) for analysis. The results were excellent for nearly all pesticide residues investigated in the fruit and vegetable matrixes except for certain pesticides that exhibited pH-dependent stability problems. Degradation of the base-sensitive pesticides captan, folpet, dichlofluanid, and chlorothalonil was observed in nonacidic matrixes, such as lettuce. This problem was reduced by the use of buffering during the extraction process, and the addition of 0.1% acetic or formic acid to the final extracts (16, 17). Based on the excellent results that have been achieved thus far with the QuEChERS method, it is being put to the test of an interlaboratory evaluation and validation study for nonfatty fruit and vegetable matrixes.

Matrix solid-phase dispersion (MSPD) is a relatively rapid, easy, and inexpensive method which has been shown to be applicable to pesticide residue analysis of fatty food matrixes (18–22). In MSPD, the sample is mixed with a sorbent, such as octadecylsilane (C_{18}) or other material commonly used in solid-phase extraction (SPE), which is placed into a column, and then an organic solvent is used to elute the analytes into a collection tube. The SPE sorbent retains certain matrix components (e.g., fat), and the extract is taken for analysis, typically by GC or LC after a solvent concentration/exchange step.

Until now, the QuEChERS method had not been tested for use with fat-containing foods, such as milk, eggs, avocado, and animal tissues. The aim of this study was to evaluate the QuEChERS method for low fatty food matrixes, and to compare it with a method developed and used by the Korean National Veterinary Research and Quarantine Service. This method is not strictly MSPD, in which the SPE sorbent is mixed with the sample using a mortar and pestle, but it is very similar to MSPD in that the sample is mixed with a drying agent prior to being transferred to an SPE column for cleanup. A comparison was also made between dispersive SPE and traditional column-based SPE for QuEChERS extracts to determine the differences in cleanup of the extracts using different sorbents. Furthermore, the effect of fat content on pesticide recoveries, depending on polarity of the analyte, was assessed.

Experimental

Instruments, Apparatus, and Chemicals

(**a**) *Analytical instruments.*—The extracts were analyzed with a Hewlett-Packard (Agilent, Little Falls, DE) 5890 Series II GC and 5972 MS instrument. Electron ionization was applied in the MS, which typically was run in the selected-ion monitoring (SIM) mode; full scan was also used in a few experiments. The system was equipped with a split/splitless injection inlet, electronic pressure control, and a 7673A autosampler. Chemstation software was used for instrument control and data analysis.

For LC/MS/MS, the extracts were concurrently analyzed with an Applied Biosystems (Toronto, Canada) API 3000 triple quadrupole instrument using electrospray ionization. The LC instrument was an Agilent 1100 with a binary pump and a Model WPALS autosampler, and Analyst software was used for instrument control and data analysis.

(**b**) *Laboratory apparatus.*—A Sorvall RT6000B centrifuge (Newtown, CT) and a Hill Scientific mv13 (Derby, CT) minicentrifuge were utilized for the 50 mL fluorinated ethylene propylene (FEP) and 2 mL centrifuge tubes (Nalgene, Rochester, NY), respectively. An Ohaus (Florham Park, NJ) GT480 top-loading balance was used to weigh the chopped samples and powder reagents. A Sartorius (Westbury, NY) R160P microbalance was used in the preparation of stock standard solutions and to weigh the tubes in experiments that determined the amount of matrix coextractives from different sample preparation conditions. A Zymark (Hopkinton, MA) Turbovap LV evaporator was employed to concentrate the extracts, when needed, and a 24-port vacuum manifold was used for SPE.

(**c**) *Reagents.*—MeCN and methanol (LC grade) were obtained from Burdick & Jackson (Muskegon, MI). Ultrapure water from a Barnstead (Dubuque, IA) water purification system was used for preparing the LC mobile phase and other aqueous solutions.

Anhydrous $MgSO_4$, anhydrous sodium acetate (NaAc), anhydrous Na_2SO_4, and NaCl, which were all ACS grade or better, were obtained from Fisher (Fair Lawn, NJ), ICN Biochemicals (Cleveland, OH), Fisher, and Mallinckrodt (Paris, KY), respectively. The $MgSO_4$ and Na_2SO_4 were baked for 5 h at 500 C in a muffle furnace to remove phthalates and residual water. Glacial acetic acid (HAc) and double-distilled formic acid (88% purity) were obtained from Mallinckrodt and GFS Chemicals (Columbus, OH), respectively. Ultrahigh purity He for GC/MS and N_2 for LC/MS/MS and solvent evaporation were obtained from Air Products (Allentown, PA).

Pesticide reference standards were obtained from the National Pesticide Standard Repository of the U.S.

Environmental Protection Agency (Fort Meade, MD), Dr. Ehrenstorfer (Augsburg, Germany), Ultra Scientific (North Kingstown, RI), and Chemservice (West Chester, PA). Stock solutions of 1000–2000 g/mL were prepared in various solvents, and working standard pesticide mixtures were prepared in MeCN. Diazinon and cyprodinil served as the internal standard (IS) in GC/MS and LC/MS/MS analyses, respectively, in egg and milk recovery experiments, and ethoprophos was the IS in the avocado experiment.

Sorbents (40 m particle size) for dispersive SPE included primary secondary amine (PSA) obtained from Varian (Harbor City, CA), C_{18} from J.T. Baker (Phillipsburg, NJ), and graphitized carbon black (GCB) from Supelco (Bellefonte, PA). SPE cartridges containing the same sorbents (500 mg) were also evaluated. For MSPD, Florisil (60–100 mesh) from Aldrich (Milwaukee, WI) was used. Eggs, whole milk, avocado, and other samples were purchased from local markets.

Analytical Methods

The buffered QuEChERS procedure consisted of the following steps: (*1*) weigh 15.0 g sample into a 50 mL FEP centrifuge tube (fortify with pesticides if the experiment requires); (*2*) add 15 mL 1% HAc in MeCN (v/v) extraction solvent into each tube (and 75 L of a 40 ng/ L IS solution to yield 200 ng/g); (*3*) add 6 g anhydrous $MgSO_4$ and 1.5 g anhydrous NaAc and shake vigorously for 1 min by hand; (*4*) centrifuge the tubes at 5000 rpm (3450 rcf) for 1 min; (*5*) transfer 1 mL MeCN extract (avoid any oily layer at the top) to a 2 mL minicentrifuge tube for dispersive SPE using 50 mg PSA + 50 mg C18 + 150 mg anhydrous $MgSO_4$; (*6*) mix the extract for 20 s and centrifuge. In an alternate cleanup step for comparison purposes, traditional column-based SPE was conducted rather than dispersive SPE.

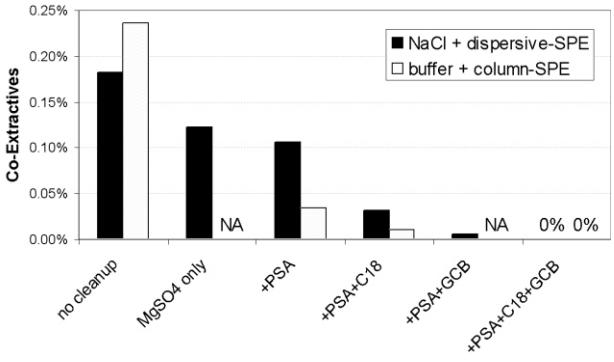

Figure 1. Comparison in terms of matrix coextractives between the use of the original QuEChERS procedure (salt-out with $MgSO_4$+NaCl) coupled with dispersive SPE and the buffered QuEChERS procedure coupled with traditional column SPE in the extraction and cleanup of homogenized egg (NA = not applicable = not done).

This simply entailed setting up a stack of C_{18} and PSA cartridges (500 mg each) in the SPE manifold, adding a 1 cm layer of anhydrous $MgSO_4$ to the top (C_{18}) cartridge, pretreating the stack with 5 mL MeCN sent to waste, and passing the extract from step 4 above through the cartridges under vacuum (ca 3 drops/s) for collection of the eluate in a 13 mL graduated centrifuge tube. No solvent evaporation steps were done for the QuEChERS extracts in either case, and final extracts were 1 g/mL sample equivalent.

In the MSPD method, 0.5 g homogenized egg or milk was thoroughly mixed with 2 g each of C_{18} Na_2SO_4 using a mortar and pestle. The sample was transferred to an empty SPE cartridge, which was stacked on the SPE manifold above a second cartridge containing 2 g Florisil. The pesticide analytes were added to the sample at this point in fortification experiments to ensure 100% transfer of analytes to the column. Then, 5 2 mL MeCN was used to elute the pesticide analytes from the sample into a 13 mL graduated centrifuge tube. A weak vacuum was applied to achieve ca 3 drops/s flow rate. The extract was concentrated to 0.5 mL by evaporation on the Turbovap at 40 C and 7.5 psi N_2 pressure, which gave a 1 g/mL sample equivalent.

In each case, the final extracts were transferred to autosampler vials for GC/MS analysis and, after calibration standards were prepared, 0.25 mL of each extract was transferred to a second autosampler vial, to which 0.75 mL of 6.67mM formic acid solution in water was added for LC/MS/MS analysis. For calibration, matrix-matched standards were prepared in blank extracts by adding the appropriate volumes of the pesticide spiking mixture and IS solution to blank extracts. An appropriate volume of MeCN was added to all vials to give consistent total volumes prior to transfer of the 0.25 mL to a second autosampler vial for LC/MS/MS analysis.

A previous publication gives the analytical conditions, retention times, and quantitation ions for the GC/MS and LC/MS/MS analyses of the pesticides (16).

Results and Discussion

Extraction and Cleanup

Lipids are not very soluble in MeCN and/or water, and the fats typically form an oily film on the surface of these solvents or an emulsion during extraction. The lipophilic pesticides remain or partition into the undissolved fats, which results in their lower recovery in the MeCN extract. However, if the fat composition of the sample is small, all or a high percentage of the fat dissolves in the MeCN and high recoveries result. This is why MeCN is not very useful for highly lipidic foods but can be used for low fatty foods. Even if recoveries are not complete in fattier foods, as long as they are consistent, an empirically-derived and well-characterized partitioning factor between the fats and the MeCN extract can be taken into account to calculate the correct concentration of the lipophilic analytes in the sample (23, 24). Depending on the capabilities of the analytical step, the limit of quantitation (LOQ) can still

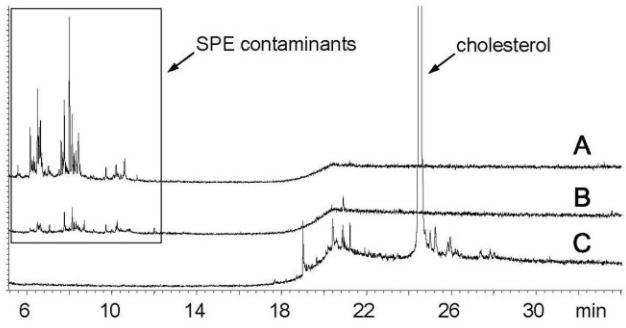

Figure 2. GC/MS full scan chromatograms of buffered QuEChERS egg extracts using column SPE for cleanup: (A) PSA + C_{18} + GCB; (B) PSA + C_{18}; and (C) PSA. A 1 cm layer of anhydrous $MgSO_4$ was added to the top cartridge of each SPE stack.

be sufficiently low in many applications for partially-recovered analytes.

Even though lipids are not very soluble in MeCN, a certain amount of fat will be coextracted, which should be removed prior to chromatographic analysis. The first step in the modification of the QuEChERS method for fatty foods was to reevaluate the effect of cleanup in the sample preparation procedure. In the case of nonfatty fruits and vegetables, the dispersive SPE cleanup step using PSA sorbent and anhydrous $MgSO_4$ alone was found to remove many matrix coextractives without affecting pesticide recoveries (14). GCB did an excellent job of removing additional matrix components from the QuEChERS extracts but, unfortunately, it also tended to remove certain pesticides, such as terbufos, thiabendazole, hexachlorobenzene, and other planar-ring analytes. The additional use of C_{18} did not affect recoveries, but neither did it provide additional cleanup over PSA alone in nonfatty matrixes.

In this study, PSA, GCB, and C_{18} sorbents were reinvestigated in both dispersive SPE and traditional SPE for cleanup of low fatty foods. Figure 1 shows the results from an experiment in which different sorbents and formats were used for cleanup of egg extracts. Also, a comparison was made between the original method, in which NaCl was used in combination with $MgSO_4$ to induce partitioning of the MeCN from water in the sample (14, 15), and the newer buffered QuEChERS procedure, in which 1% HAc is added to the MeCN for extraction and NaCl is replaced by NaAc to yield consistent pH of the procedure independent of the pH of the original sample (16, 25). As the figure shows, the buffered procedure coextracted somewhat more matrix components in the case of eggs than the original procedure, but the SPE cleanup approaches overcame this difference. The buffered QuEChERS modification has advantages with respect to higher recoveries and greater stability of pH-sensitive pesticides (16); thus, it was used in all other experiments. $MgSO_4$ alone (150 mg/mL extract) in dispersive SPE provided some cleanup by removing residual water (and possibly other components via chelation). The addition of 50 mg PSA/mL extract along with the $MgSO_4$ did not provide much additional cleanup in dispersive SPE, but the column-based method, in which 10 mL extract was passed through a 500 mg PSA cartridge topped by 1 cm $MgSO_4$, provided a 6-fold reduction in the amount of coextractives. As also shown previously in the case of eggs (13), C_{18} provided excellent cleanup in both column SPE and dispersive SPE, and the addition of GCB essentially eliminated all coextractives that could be measured by weight difference in 5 mL of extracts by either SPE format. Figure 2 further shows chromatograms indicating how C_{18} removes the large cholesterol component from the egg extracts, verifying the weight-based measurements that no significant interferences remain from the egg when C_{18} and GCB are used in cleanup. Unfortunately, the SPE cartridges add relatively volatile interferences to the extracts, as the chromatograms show, especially in the case of GCB, which strongly adsorbs chemicals from the atmosphere. We found that pretreatment of the SPE cartridges with toluene was needed to remove these contaminants.

Despite the excellent cleanup provided by the combination of all 3 sorbents plus $MgSO_4$, the problem remained that planar-ring pesticides (e.g., hexachlorobenzene) were strongly retained by GCB, and even straight toluene did not elute those analytes completely (16). Furthermore, when toluene was employed in elution from GCB, the cleanup was minimal; thus, GCB was not used in the final method. However, GCB may be employed for cleanup if the list of targeted analytes in a particular application does not include planar-ring pesticides. Otherwise, the combination of PSA + C_{18} was shown to be very effective for the cleanup of egg extracts (*see* Figures 1 and 2), and traditional column SPE in the buffered approach was found to remove somewhat more matrix components from egg extracts than dispersive SPE.

Method Comparison and Evaluation

Because the dispersive SPE procedure requires only 1 mL extract, there was enough remaining for ca 10 mL of the same buffered QuEChERS extract to be used in column SPE cleanup when fortification experiments were conducted; this conveniently afforded direct comparison of the pesticide recoveries between the 2 formats. In the column SPE format, the extract is simply passed through the cartridge stack in an SPE vacuum manifold and collected in a tube. This is a form of "chemical filtration," in which matrix components are retained and pesticides pass through the sorbents, while the MeCN serves as both the extract medium and elution solvent.

Table 1 presents the results from the fortification experiment of whole milk and homogenized egg using the QuEChERS method with the different SPE cleanup formats and the MSPD method. As the table shows, the results for the wide range of 30 pesticides at low (50 ng/g) and high (500 ng/g) spiking levels in both the milk and egg matrixes generally fell within the commonly accepted range of 70–120% recovery and 15% relative standard deviation

Table 1. Average %recoveries (%RSD) of fortified pesticides in milk and egg at different concentrations from the buffered QuEChERS method with dispersive SPE and column SPE cleanup, and the MSPD method with GC/MS and LC/MS/MS analyses

	QuEChERS[a]											
	Dispersive SPE				Column SPE				MSPD[a]			
	Milk		Egg		Milk		Egg		Milk		Egg	
Pesticide	50 ng/g (*n* = 3)	500 ng/g (*n* = 3)	50 ng/g (*n* = 3)	500 ng/g (*n* = 3)	50 ng/g (*n* = 3)	500 ng/g (*n* = 3)	50 ng/g (*n* = 3)	500 ng/g (*n* = 3)	50 ng/g (*n* = 3)	500 ng/g (*n* = 3)	50 ng/g (*n* = 3)	500 ng/g (*n* = 3)
Acephate[b]	107 (3)	101 (8)	107 (3)	107 (6)	105 (2)	109 (1)	103 (5)	**49 (26)**	98 (1)	112 (7)	77 (4)	**66 (22)**
Captafol	**ND**	97 (15)	**ND**	**66** (2)	**ND**	120 (15)	**ND**	97 **(62)**	**ND**	**123** (13)	**ND**	97 (13)
Captan	108 (8)	105 (7)	**ND**	71 (9)	95 (8)	108 (7)	**ND**	**68 (37)**	**ND**	120 (10)	**ND**	74 (7)
Carbaryl[b]	112 (4)	114 (6)	118 (1)	**124** (2)	113 (1)	115 (2)	**126** (2)	**123** (2)	105 (1)	113 (4)	101 (2)	104 (2)
Carbendazim[b]	93 (4)	105 (5)	92 (3)	**139** (2)	88 (2)	103 (4)	72 (5)	**66** (7)	94 (2)	**123** (10)	**66 (32)**	**127** (1)
Chlordane	80 (4)	85 (5)	74 (6)	74 (3)	81 (7)	79 (6)	**67** (4)	**69** (8)	114 (6)	102 (9)	102 (7)	101 (3)
Chlorothalonil	**135 (18)**	91 (6)	**64 (16)**	**68** (3)	**159** (9)	100 (4)	**ND**	90 **(86)**	110 **(36)**	99 (9)	**ND**	**ND**
Chlorpyrifos	100 (1)	94 (4)	87 (8)	90 (3)	95 (1)	93 (2)	93 (4)	90 (2)	116 (2)	100 (5)	110 (5)	104 (3)
Chlorpyrifos-methyl	99 (2)	99 (3)	96 (6)	95 (2)	101 (3)	97 (3)	101 (4)	98 (1)	115 (1)	100 (3)	103 (5)	99 (1)
Coumaphos	117 (5)	114 (6)	**124 (18)**	113 (7)	101 (4)	108 (12)	**152 (33)**	**131** (8)	**150** (5)	**126** (6)	**128** (3)	**121** (8)
Cyprodinil	96 (5)	96 (6)	93 (14)	89 (4)	98 (5)	85 (4)	90 (9)	90 (4)	**125** (4)	113 (2)	102 (4)	111 (6)
DDE	70 (4)	75 (5)	**62** (12)	**63** (5)	72 (8)	70 (6)	**56** (6)	**56** (9)	**121** (6)	102 (9)	96 (9)	102 (6)
Dichlofluanid[b]	91 (2)	105 (2)	**31 (20)**	79 (4)	93 (1)	113 (2)	**29 (26)**	70 **(19)**	**58** (2)	104 (3)	**31 (16)**	**56 (28)**
Dichlorobenzophenone	75 (7)	94 (10)	84 (6)	82 (7)	83 (8)	85 (7)	78 (7)	80 (8)	**127** (1)	116 (6)	**132** (8)	114 (7)
Dichlorvos	111 (7)	115 (5)	105 (12)	106 (3)	110 (5)	107 (3)	117 (8)	115 (5)	**67 (38)**	96 (10)	83 (11)	86 **(20)**
Dieldrin	88 (3)	95 (5)	87 (9)	79 (2)	83 (8)	86 (5)	76 (8)	76 (5)	**133** (15)	100 (10)	98 (13)	103 (5)
Endosulfan sulfate	110 (12)	102 (6)	101 (13)	105 (5)	105 (10)	101 (3)	119 **(16)**	112 (5)	**155** (5)	119 (4)	120 (11)	106 (6)
Folpet	113 (1)	94 (6)	**51 (17)**	74 (2)	92 (9)	100 (8)	**ND**	77 **(42)**	97 **(87)**	110 (8)	101 **(26)**	84 (3)
Heptachlor epoxide	86 **(17)**	97 (3)	93 (9)	80 (2)	91 (13)	89 (6)	89 **(16)**	81 (3)	**127** (9)	101 (7)	105 (5)	101 (4)
Hexachlorobenzene	**62** (8)	**66** (4)	**45** (9)	**45** (8)	**62** (8)	**60** (9)	34 (10)	**36 (20)**	74 (11)	**65** (8)	75 (12)	81 (6)
Imazalil[b]	80 (5)	83 (4)	96 (1)	97 (2)	86 (2)	90 (3)	75 **(19)**	**56 (69)**	**4 (27)**	1 (14)	**4 (21)**	**ND**
Imidacloprid[b]	113 (3)	119 (7)	117 (2)	**122** (4)	113 (1)	116 (1)	**128** (3)	**130** (4)	107 (5)	**121** (7)	104 (4)	105 (2)
Lindane	99 (7)	106 (4)	93 (9)	95 (0)	91 (3)	97 (4)	96 (5)	94 (1)	114 (2)	97 (5)	96 (6)	96 (3)
Methamidophos[b]	106 (7)	109 (5)	93 (5)	100 (6)	105 (5)	85 (6)	77 (6)	77 (12)	96 **(21)**	113 **(19)**	81 **(16)**	92 (11)
Penconazole[b]	99 (2)	101 (1)	106 (1)	108 (2)	104 (0)	108 (2)	108 (1)	107 (7)	90 (2)	79 **(19)**	**68 (25)**	72 (7)
Permethrins	86 (8)	88 (10)	86 (12)	78 (6)	84 (3)	78 (6)	79 (15)	80 (5)	**132** (10)	112 (6)	112 (6)	114 (6)

Table 1. (*continued*)

Pesticide	QuEChERS[a]						MSPD[a]			
	Dispersive SPE				Column SPE					
	Milk		Egg		Milk		Egg		Milk		Egg	
	50 ng/g (*n* = 3)	500 ng/g (*n* = 3)	50 ng/g (*n* = 3)	500 ng/g (*n* = 3)	50 ng/g (*n* = 3)	500 ng/g (*n* = 3)	50 ng/g (*n* = 3)	500 ng/g (*n* = 3)	50 ng/g (*n* = 3)	500 ng/g (*n* = 3)	50 ng/g (*n* = 3)	500 ng/g (*n* = 3)
Propoxur	118 (5)	110 (3)	**124** (3)	**121** (6)	113 (7)	110 (3)	**125 (26)**	**138 (27)**	**160** (14)	110 (7)	**155 (16)**	**122** (5)
Pymetrozine[b]	98 (2)	106 (1)	93 (4)	114 (5)	83 (1)	80 (3)	72 (7)	**67 (29)**	**18** (6)	**10 (30)**	**11** (14)	**2 (23)**
Thiabendazole[b]	111 (1)	111 (2)	**131** (0)	118 (2)	103 (2)	104 (2)	103 (2)	87 **(22)**	**8** (13)	**5 (110)**	**16 (54)**	**13 (69)**
Tolylfluanid[b]	93 (1)	106 (2)	**40** (12)	88 (2)	98 (2)	115 (2)	**41 (16)**	84 (11)	75 (3)	110 (3)	**46** (13)	**67 (20)**

[a] ND = Not detected; bold numbers indicate values not meeting the criteria of 70–120% recovery and 15% RSD.

[b] LC/MS/MS result.

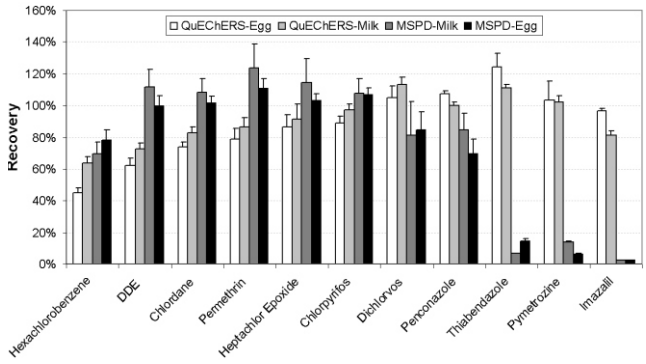

Figure 3. Comparison of the buffered QuEChERS method with dispersive SPE and MSPD methods for the extraction of selected pesticides fortified at 50 and 500 ng/g in egg and milk matrixes (*n* = 6). Error bars signify standard deviation.

(RSD) for quantitative pesticide residue methods (26). Some method validation guidelines expand this acceptable range to 50–150% recovery and 20% RSD (27), and nearly all analytes would have met these looser criteria. The results for those pesticides marked by a superscript b were from the LC/MS/MS analysis, and the others are from GC/MS. When the analyte was measured by both techniques, both results were usually similar, but generally the LC/MS/MS result is deemed more trustworthy (15, 16).

In comparing the dispersive SPE cleanup with the column SPE format, mostly insignificant differences occurred except for a few interesting examples (mostly in the case of egg). These differences consist of those pesticides with acid/base properties (acephate, carbendazim, imazalil, methamidophos, pymetrozine, and thiabendazole). The dispersive SPE approach yielded higher and more consistent recoveries of these analytes than the traditional SPE procedure. This was previously observed in the case of acephate, which is one reason why the dispersive SPE method was chosen over traditional SPE in the QuEChERS approach (14, 16). Other reasons are that dispersive SPE is easier, cheaper, faster, and requires less materials and equipment than the cartridge-based format. The slightly better cleanup provided by column SPE does not compensate for the better recoveries and practical advantages of dispersive SPE.

Figure 3 graphically shows the results from this experiment for those pesticides that gave interesting differences between the QuEChERS method with dispersive SPE and the MSPD method. The MSPD fortification experiments, as conducted, performed somewhat better for those lipophilic analytes on the left side of the graph (hexachlorobenzene, DDE, and chlordane), and the QuEChERS method performed much better for the basic pesticides on the right side of the graph (imazalil, pymetrozine, and thiabendazole). The pesticides shown in the middle had relatively small differences, and the many pesticides not shown had insignificant differences (*see*

Table 2. Average % recoveries (%RSD) of fortified pesticides in avocado from the buffered QuEChERS method with dispersive SPE and GC/MS and LC/MS/MS analysis

Pesticide	50 ng/g[a] (*n* = 3)	250 ng/g[a] (*n* = 3)	Overall[a] (*n* = 6)
Acephate[b]	107 (1)	108 (4)	108 (3)
Carbaryl[b]	108 (0)	106 (1)	107 (1)
Carbendazim[b]	108 (2)	104 (2)	106 (3)
Chlordane	**51** (4)	**54** (7)	**52** (6)
Chlorothalonil	94 (10)	91 (14)	92 (10)
Chlorpyrifos	82 (1)	79 (4)	80 (3)
Chlorpyrifos-methyl	86 (2)	88 (3)	87 (2)
Coumaphos	95 (11)	99 (3)	97 (7)
Cyprodinil[b]	75 (2)	77 (3)	76 (3)
DDE	**37** (2)	**38** (10)	**38** (6)
Diazinon	91 (1)	94 (1)	93 (2)
Dichlofluanid[b]	93 (2)	99 (1)	96 (4)
Dichlorobenzophenone	**66** (7)	**64** (6)	**65** (6)
Dichlorvos	95 (5)	106 (2)	101 (7)
Dieldrin	**56** (8)	**64** (7)	**60** (9)
Endosulfan sulfate	89 (3)	90 (10)	90 (6)
Heptachlor epoxide	**66** (12)	**66** (4)	**66** (7)
Hexachlorobenzene	**27** (2)	**28** (7)	**27** (5)
Imazalil[b]	92 (1)	89 (1)	90 (2)
Imidacloprid[b]	112 (0)	111 (1)	111 (1)
Lindane	77 (7)	80 (6)	78 (6)
Methamidophos[b]	103 (3)	103 (4)	103 (3)
Penconazole[b]	96 (1)	94 (2)	95 (2)
Permethrins	**60** (7)	**64** (7)	**62** (7)
Propoxur	101 (9)	106 (7)	103 (7)
Pymetrozine[b]	100 (1)	103 (3)	102 (3)
Thiabendazole[b]	104 (2)	100 (1)	102 (3)
Thiophanate-methyl[b]	102 (3)	117 (3)	110 (7)
Tolylfluanid[b]	91 (2)	96 (1)	94 (3)

[a] *See* footnote a in Table 1.
[b] LC/MS/MS result.

Table 1). The permethrin result gave a 10–20% high bias in MSPD and, in the same approach, penconazole gave a lower recovery than other analytes of a similar nature. Periodic high biases also curiously occurred in the methods for certain analytes (carbaryl, coumaphos, propoxur, and imidacloprid), which was believed to be due to calibration issues.

The ca 20% lower recovery and greater variability for the most volatile analyte, dichlorvos, in the MSPD method was a result of losses during the solvent evaporation step in the method. The very low recoveries of the basic pesticides (thiabendazole, imazalil, pymetrozine, and, to a lesser extent, penconazole) in the MSPD method probably relate to the Florisil cleanup step. In addition to their fungicidal properties, imazalil and thiabendazole are also veterinary drugs (an antimycotic and anthelmintic, respectively); thus, it is worthwhile that they be included in monitoring methods for milk and other animal-derived foods (28).

In the case of the most lipophilic pesticides spiked into the matrixes, MSPD gave up to 50% higher recoveries than the QuEChERS method in milk and egg. In MSPD, the fatty tissues are mechanically ruptured by the excess solid crystals, and the extraction solvent may have better access to the analytes contained in the fat. Of course, incurred samples were not extracted in this experiment and spiking was done in the tubes, not in the mortar, thus it is difficult to assess this factor based on these results.

Effect of Fat Content on Pesticide Recoveries

Even in this study, MSPD using MeCN gave lower recovery (70–80%) of hexachlorobenzene, the most lipophilic analyte evaluated, than other analytes in milk and egg. In the case of the QuEChERS method, the recovery was even lower, and a stronger relationship between recovery and fat content was observed. Whole milk contains 3.25% fat and raw eggs are 9.94 0.14% fat, based on $n = 23$ (10). Table 2 shows the pesticide recoveries of avocado, an even fattier sample matrix with 14.66 0.54% fat based on $n = 35$ (10); the same trend is observed for lipophilic pesticides, but to a greater extent, due to the higher fat content.

Figure 4 exhibits this trend in the QuEChERS recoveries of those pesticides tested that were affected by fat content in different matrixes. In the case of nonfatty matrixes, the combined lettuce and orange results ($n = 36$) from the buffered QuEChERS method reported previously (16) are given in the figure. Otherwise, the overall results from the milk, egg, and avocado ($n = 6$ each) are shown. A trend in recoveries versus % fat in the sample can be observed. The extent of the effect depends on the analyte partitioning coefficient between the lipid film and the MeCN phase upon which it floats in the initial extract. This partitioning can be estimated by the octanol–water coefficient (K_{ow}) of the pesticide, but this value is difficult to measure and reports in the literature are rather variable, especially for the most nonpolar and polar analytes. Plots of pesticide recoveries versus K_{ow} in this study did not give a clear picture of the relationship. The measurement of pesticide solubility in water is easier and more accurate than K_{ow}, and this has been shown previously to be a good indicator in the evaluation of polarity range (analytical scope) and suitability of pesticide methods for different applications (29). Figure 5 shows the relationship between pesticide recoveries by the MeCN extraction procedure versus their reported solubilities in water (30) and fat content in the food. The least water-soluble pesticide evaluated was permethrin, appearing farthest to the left in Figure 5 which, interestingly, is not the most lipophilic analyte. Like most pyrethroids, permethrin has relatively higher solubility and greater affinity for MeCN than do other nonpolar pesticides, which counteracts its lipophilicity to some extent (24).

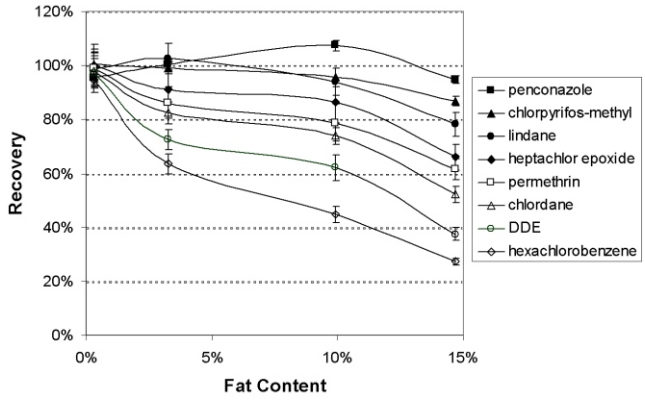

Figure 4. Effect of fat content in the sample on selected pesticide recoveries for the buffered QuEChERS method with dispersive SPE. The commodities consisted of lettuce and orange (*n* = 36), 0.3% fat; whole milk (*n* = 6), 3.25% fat; homogenized egg (*n* = 6), 9.9% fat; and avocado (*n* = 6), 14.7% fat. Error bars signify standard deviation.

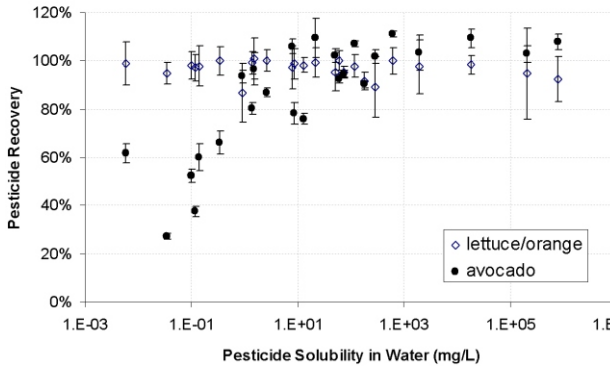

Figure 5. The effect of water solubility (polarity) on pesticide recoveries using the buffered QuEChERS method with different commodities, depending on fat content: lettuce/orange (*n* = 36) with 0.3% fat and avocado (*n* = 6) with 14.7% fat. Error bars signify standard deviation.

Otherwise, Figure 5 in the case of avocado shows a rather clear relationship between the recovery of pesticides and their solubility in water. Analytes with water solubility > ca 0.5 mg/L gave >70% recovery for the 15% fat matrix, whereas the 70% recovery cutoff for egg (10% fat) among nonpyrethroids was ca 0.3 mg/L, and ca 0.1 mg/L for whole milk (3% fat). In nonfatty matrixes, the entire pesticide polarity range from pyrethroids (0.001 mg/L) to acephate (790 000 mg/L) in terms of water solubility was covered quantitatively by the simple and effective method.

Conclusions

The buffered QuEChERS method with dispersive SPE was the simplest and fastest of the sample preparation approaches tested in this study. The MSPD-like method used by the Korean National Veterinary Research and Quarantine Service was slightly less expensive in terms of material costs, but it had lower sample throughput because the sample mixing step could only be done sequentially by hand, and the column-SPE procedure and solvent evaporation steps took more time than dispersive SPE. Also, the MSPD method needed a few more pieces of glassware and laboratory devices than did the QuEChERS method, which only entailed washing an FEP centrifuge tube afterwards. Each method had advantages over the other in terms of analytical scope in that the MSPD method had somewhat higher recoveries of the most lipophilic pesticides fortified in the case of milk and eggs, whereas the QuEChERS method gave complete recoveries of basic pesticides that were barely recovered by the MSPD method. The final extracts were the same concentration in both methods; thus, they each essentially gave equivalent LOQs, depending on recoveries. Even though nonpolar OCs, such as hexachlorobenzene, DDE, and chlordane, gave 25–50% recovery by the QuEChERS method in avocado, the LOQs (signal-to-noise ratio = 10) were extrapolated to be <10 ng/g in GC/MS (SIM) with 1 L injections of the 1 g/mL equivalent extracts. Furthermore, the recoveries were very consistent for the samples tested, thus compensation could be made for the reduced recoveries in the calculated results after further testing and validation. In any case, analysis of incurred reference materials or proficiency testing should be done to provide more trustworthy conclusions, particularly about the extractability of lipophilic pesticides. Although the QuEChERS method is not likely to be applicable to extraction of lipophilic pesticides in high fatty samples, it is acceptable for their extraction from low fatty foods and for the extraction of polar and semipolar pesticides from a wide variety of fatty foods. It remains very useful for the complete extraction of a very wide polarity range of pesticides from nonfatty foods.

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References

(1) U.S. Food and Drug Administration (1994) *Pesticide Analytical Manual Vol. I, Multiresidue Methods,* 3rd Ed., U.S. Department of Health and Human Services, Washington, DC

(2) Di Muccio, A. (2000) "Organochlorine, Pyrethrin and Pyrethroid Insecticides; Single Class, Multiresidue Analysis

of " in *Encyclopedia of Analytical Chemistry Applications Theory and Instrumentation*, R.A. Meyers (Ed.), John Wiley & Sons, New York, NY, Vol. 7, pp 6384–6420

(3) Food Safety Inspection Service (1991) *Analytical Chemistry Residue Laboratory Guidebook, Residue Chemistry* CHC3 Method, U.S. Department of Agriculture, Washington, DC

(4) Holstege, D.M., Scharberg, D.L., Tor, E.R., Hart, L.C., & Galey, F.D. (1994) *J. AOAC Int.* **77,** 1263–1274

(5) Maštovská, K. (2005) "Food & Nutritional Analysis: (q) Pesticide Residues," in *Encyclopedia of Analytical Science,* 2nd Ed., P. Worsfold, A. Townshend, & C. Poole (Eds), Elsevier, Oxford, UK, Vol. 3, pp 251–260

(6) Zrostlíková, J., Lehotay, S.J., & Hajšlová, J. (2002) *J. Sep. Sci.* **25**, 527–537

(7) van der Hoff, G.R., van Beuzekom, A.C., Brinkman, U.A., Baumann, R.A., & van Zoonen, P. (1996) *J. Chromatogr. A* **754**, 487–496

(8) Food Safety Inspection Service (2003) *2000 National Residue Program Data*, U.S. Department of Agriculture, Washington, DC (www.fsis.usda.gov/Frame/FrameRedirect.asp?main=/oppde/ric/links.htm)

(9) Agricultural Marketing Service (2003) *Pesticide Data Program Annual Summary Calendar Year 2001*, U.S. Department of Agriculture, Manassas, VA (www.ams.usda.gov/science/pdp/)

(10) Agricultural Research Service (2004) *USDA Nutrient Database for Standard Reference,* Release 16-1, U.S. Department of Agriculture, Beltsville, MD (www.nal.usda.gov/fnic/foodcomp)

(11) Bennett, D.A., Chung, A.C., & Lee, S.M. (1997) *J. AOAC Int.* **80**, 1065–1077

(12) Sheridan, R.S., & Meola, J.R. (1999) *J. AOAC Int.* **82**, 982–990

(13) Lehotay, S.J., Lightfield, A.R., Harman-Fetcho, J.A., & Donoghue, D.J. (2001) *J. Agric. Food Chem.* **49**, 4589–4596

(14) Anastassiades, M., Lehotay, S.J., Štajnbaher, D., & Schenck, F.J. (2003) *J. AOAC Int.* **86**, 412–431

(15) Lehotay, S.J., de Kok, A. Hiemstra, M., & van Bodegraven, P., (2005) *J. AOAC Int.* **88**, 595–614

(16) Lehotay, S.J., Maštovská, K., & Lightfield, A.R. (2005) *J. AOAC Int.* **88**, 615–629

(17) Maštovská, K., & Lehotay, S.J. (2004) *J. Chromatogr. A* **1040**, 259–272

(18) Long, A.R., Soliman, M.M., & Barker, S.A. (1991) *J. Assoc. Off. Anal. Chem.* **74**, 493–496

(19) Barker, S.A. (2000) *J. Chromatogr. A* **885**, 115–127

(20) Barker, S.A. (2000) *J. Chromatogr. A* **880**, 63–68

(21) Schenck, F.J., & Wagner, R. (1995) *Food Addit. Contam.* **12**, 535–541

(22) Di Muccio, A., Pelosi, P., Camoni, I., Attard Barbini, D., Dommarco, R., Generali, T., & Ausili, A. (1996) *J. Chromatogr. A* **754**, 497–506

(23) Bowman, M.C., & Beroza, M. (1965) *J. Assoc. Off. Anal. Chem.* **48**, 943–952

(24) Argauer, R.J., Lehotay, S.J., & Brown, R.T. (1997) *J. Agric. Food Chem.* **45**, 3936–3939

(25) Lehotay, S.J. (in press) "Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) Approach for Determining Pesticide Residues," in *Methods in Biotechnology, Pesticide Analysis*, J.L. Martinez-Vidal & G. Frenich (Eds), Humana Press, New York, NY

(26) Hill, A.R., & Reynolds, S.L. (1999) *Analyst* **124**, 953–958

(27) Agricultural Marketing Service (2002) *Acceptability Criteria of Process Control and Fortification Recoveries*, PDP-QC-04, U.S. Department of Agriculture, Manassas, VA (www.ams.usda. gov/science/pdp/Qc04.pdf)

(28) U.S. Environmental Protection Agency, *Code of Federal Regulations, Title 40, Subsection E, Pesticide Programs Part 180,* U.S. Government Printing Office, Washington, DC (www.epa.gov/pesticides/food/ viewtols.htm)

(29) Lehotay, S.J., & Lee, C.-H. (1997) *J. Chromatogr. A* **785**, 313–327

(30) *The Pesticide Manual*, 12th Ed. (2000) C.D.S. Tomlin (Ed.), The British Crop Protection Council, Surrey, UK