The Matrix Effects of 88 Pesticide Residues in 19 Types of Fruits and Vegetables by GC-MS/MS

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Abstract [**Objectives**] This study was conducted to address the fragmentation of existing research on matrix effects in pesticide residue detection by gas chromatography-tandem mass spectrometry (GC-MS/MS) and to overcome the limitations of the single-matrix single-pesticide research model. [**Methods**] A comprehensive matrix effect evaluation matrix covering "88 pesticides × 19 fruit and vegetable matrices" was constructed for the first time. The interference patterns of fruit and vegetable matrices on pesticide residue detection were systematically investigated, and representative matrices were screened for each matrix effect gradient. [**Results**] Among the tested pesticides, 95.8% exhibited matrix enhancement effects ($ME > 100\%$), while 4.2% showed matrix suppression effects. Among these, medium matrix enhancement effects in the range of 120% - 150% accounted for the highest proportion, reaching 54.9%. Six pesticides including paclobutrazol and terbufos showed significant matrix enhancement effects in 18 matrices except bean sprout, while deltamethrin, tetrachlorvinphos, and edifenphos exhibited matrix suppression characteristics in most matrices. Meantime, bean sprout, cucumber, mango, carrot, and cowpea can serve as typical representative matrices for the relatively weak, weak, medium, strong, and very strong matrix enhancement intervals, respectively. [**Conclusions**] This study has clarified the matrix effect characteristics in a multi-pesticide, multi-fruit/vegetable system, providing a theoretical basis and technical support for high-throughput and accurate detection of pesticide residues in fruits and vegetables. It holds significant application value for food safety risk monitoring and regulatory work.

Key words GC-MS/MS; Matrix effect; Pesticide residues; Fruits and vegetables

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Pesticides have chronic toxicity and can affect the human nervous, reproductive, and immune systems. Long-term exposure to pesticides can be carcinogenic, teratogenic, and mutagenic. At present, China ranks first in the world in terms of pesticide application amount in crops, and over 800 types of pesticide residues have been detected in vegetables and fruits. In recent years, pesticide residues exceeding the maximum residue limits (MRLs) have ranked top among the substances identified in national food safety sampling inspections. The national compliance rates for MRLs in vegetables and fruits were only 58.7% and 63.2%, respectively[1]. Therefore, the supervision and control of pesticide residues in the food safety field still face a long and arduous task ahead. Although China has issued GB 2763-2026 judgment standard and the "three chromatography + two mass spectrometry" five-standard system as supporting documents for plant-derived food safety supervision, the accuracy and efficiency of pesticide residue detection are still limited due to the limitation of matrix effect of mainstream mass spectrometry detection technology[2]. Therefore, how to address and properly manage the widespread problem of matrix effects in high-throughput detection has become one of the most valuable research directions in pesticide residue analysis.

At present, liquid chromatography-tandem mass spectrometry (LC-MS/MS) is one of the mainstream methods for high-throughput screening of pesticide residues. However, gas chromatography-tandem mass spectrometry (GC-MS/MS) remains an irreplaceable and important complementary tool. The two are not in competition but coexist as complementary techniques. In official methods of various countries (such as China's GB, the EU SANTE guidelines, and the US FDA PAM), a large number of methods are still based on GC-MS/MS. Therefore, modern pesticide residue testing laboratories generally adopt a "dualplatform combined" strategy. The currently effective national food safety standards for pesticide residue detection, such as GB 23200.113-2026 and GB 23200.8-2016, explicitly require that when GC-MS is used to determine pesticide residues in fruits and vegetables, matrixmatched standard solutions must be adopted for quantitative analysis. Although many studies have been conducted both at home and abroad to investigate the matrix effects in the determination of pesticide residues in various matrices by GC-MS[3-4]. However, existing related studies still have obvious limitations. Most research has focused only on a single vegetable or fruit matrix system, resulting in limited coverage of detectable matrices[5-6] and a narrow range of target pesticide categories[7-9]. In addition, although many studies have used mixed matrices to cancel out matrix effects between different fruit and vegetable matrices[10-12], research on matrix similarity effects remains scarce[13-14]. A systematic comparative analysis system for matrix effects has not yet been established, nor has a stratified screening and classification study of typical matrices been completed. Based on this, the present study focuses on the shortcomings of existing technologies. Nineteen common types of fruits and vegetables belongin to ten categories were selected as research matrices, and 88 typical pesticide residues were chosen as target analytes. The differences in matrix effects among various matrices were systematically investigated using GC-MS/MS. Through statistical analysis, representative fruit and vegetable detection matrices were identified. This study aims to provide reliable data support and a theoretical basis for the

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mitigation and correction of matrix effects in multipesticide residue analysis of fruits and vegetables. It is helpful to improve the detection efficiency and accuracy of batch screening of pesticide residues in fruits and vegetables, and has important theoretical significance and application value for improving the food safety risk monitoring system and building a solid food safety defense line for fruits and vegetables.

Materials and Methods

Reagents and consumables

Test vegetables: Eleven types of vegetables were selected, including leaf vegetables (celery, romaine lettuce, Shanghai bok choy), root vegetables (carrot), bulb vegetables (scallion, Chinese chive), sprout vegetables (bean sprout), solanaceous vegetables (pepper), gourd vegetables (cucumber), legume vegetables (cowpea), and root/tuber vegetables (ginger). Eight types of fruits were selected, including Citrus fruits (tangerine, sugar mandarin, pomelo, orange, sweet orange), and tropical and subtropical fruits (banana, dragon fruit, mango). All vegetables and fruits were purchased from a supermarket in Changsha City and were tested negative for the target pesticides.

Eighty-eight pesticide reference standards (Table 1) with purities >98% were obtained from China Tanmo Quality Inspection Technology Co., Ltd.

The internal standard, heptachlor epoxide B, was obtained from China Tanmo Quality Inspection Technology Co., Ltd. Acetonitrile (chromatographic grade) was purchased from Shanghai Anpel Experimental Technology Co., Ltd. Acetone (chromatographic grade) was purchased from Shanghai Anpel Experimental Technology Co., Ltd. Ethyl acetate (chromatographic grade) was purchased from Shanghai Anpel Experimental Technology Co., Ltd.

Other reagents and consumables used were as follows: QuEChERS extraction salt packet containing 0.5 g of hydrogen citrate, obtained from Shanghai Anpel Experimental Technology Co., Ltd.; ceramic homogenizer (model JK-TCJZZ50), obtained from Weifang Jukai Electronic Technology Co., Ltd.; QuEChERS purification tube 1, containing 900 mg of magnesium sulfate and 150 mg of PSA, obtained from Qingyun Chemical Technology Co., Ltd.; QuEChERS purification tube 2, containing 885 mg of magnesium sulfate, 150 mg of PSA, and 15 mg of GCB, obtained from Qingyun Chemical Technology Co., Ltd.; organic phase syringe filter (13 mm×0.22 μm), obtained from Shanghai Anpel Experimental Technology Co., Ltd.; and centrifuge tubes (50 ml), obtained from Hunan Zhongke Hongsheng Instrument Co., Ltd.

Instruments and equipment

GC-MS/MS (GC-MS/MS): model 8890-7000D, Agilent Technologies, Inc.; electronic balance: model PL303, Mettler Toledo Technologies (China) Co., Ltd.; centrifuge: model 3K15, Sigma-Aldrich (Shanghai) Trading Co., Ltd.; vortex mixer: model YM-H8, Hunan Michael Laboratory Instrument Co., Ltd.; shaker: model SA 320, Yamato Corporation, Japan; pipettes: 100 μl/1 000 μl, FINNPIPETTE F3, Thermo Fisher Scientific (Shanghai) Co., Ltd.

Sample pretreatment method

A 10 g portion of each sample was accurately weighed (accurate to 0.01 g) and added into a 50 ml plastic centrifuge tube. Next, 10 ml of acetonitrile, the QuEChERS salt packet, and one ceramic homogenizer were added. The tube was capped, vortexed for 30 s, shaken vigorously for 10 min, and centrifuged at 8 000 r/min for 2 min. Next, a 6 ml aliquot of the supernatant was transferred into a commercial QuEChERS purification tube, vortexed for 1 min, and centrifuged at 6 000 r/min for 1 min. A 2 ml aliquot of the supernatant was accurately transferred into a 10 ml test tube and evaporated to near dryness under a nitrogen stream in a 40 ℃ water bath. Subseqently, 20 μl of a 5 mg/L heptachlor epoxide B internal standard solution and 1 ml of ethyl acetate were added to redissolve the residue. The resulting solution was filtered through a microporous membrane to obtain the blank matrix solution of the vegetable or fruit. The blank matrix solutions prepared from multiple parallel samples were mixed together and were then ready for the preparation of matrixmatched standard solutions.

Samples purified with purification tube 1 mainly included bean sprout, ginger, cucumber, tangerine, sugar mandarin, pomelo, orange, sweet orange, and banana. Samples purified with purification tube 2 mainly included dark-colored samples, such as scallion, Chinese chive, pepper, cowpea, romaine lettuce, celery, carrot, Shanghai bok choy, dragon fruit, and mango.

Preparation of standard solutions

Preparation of reference substances: Each pesticide standard was dissolved in acetonitrile or acetone to prepare individual stock solutions at a concentration of 1 000 mg/L. Appropriate aliquots of these stock solutions were then transferred into the same 10 ml volumetric flask and diluted with acetonitrile to obtain a mixed standard solution at a concentration of 5 mg/L, which was then stored at −18 ℃ for later use. An aliquot of the 1 000 mg/L heptachlor epoxide B internal standard solution was taken and diluted with ethyl acetate stepwise to prepare an internal standard working solution at a concentration of 5 mg/L for later use. An aliquot of the 1 000 mg/L heptachlor epoxide B internal standard solution was diluted stepwise with ethyl acetate to prepare an internal standard working solution at a concentration of 5 mg/L for later use. Subsequently, the mixed standard solution was stepwise diluted with ethyl acetate, and 20 μl of the 5 mg/L heptachlor epoxide B internal standard solution was added to each dilution to prepare solvent standard solutions with an internal standard concentration of 100 ng/ml and calibration curve series of 10, 20, 40, 80, 120, and 200 ng/ml.

Preparation of solutions for matrix effect evaluation: Matrix-matched standard solutions at three concentration levels (20, 60, and 120 ng/ml) were prepared using the blank sample extracts of eleven vegetables and eight fruits. Five replicate solutions were made for each matrix. The matrix effect was evaluated by compa-

ring the relative peak area ratios of the target compounds obtained from the matrixmatched standard solutions with those obtained from the solvent standard solutions.

GC-MS/MS analytical method

Chromatographic conditions The chromatographic separation was performed on an Agilent HP-5MS UI column (30 m×0.25 mm ×0.25 μm). The column oven temperature was programmed as follows: holding at 70 ℃ for 2 min, then increasing to 200 ℃ at a rate of 20 ℃/min, further increasing to 300 ℃ at a rate of 40 ℃/min, and holding at 300 ℃ for 4 min. The injector temperature was set at 280 ℃, and the transfer line temperature was set at 280 ℃. Helium with a purity of ≥99.999% was used as the carrier gas at a flow rate of 1 ml/min. The injection was performed in splitless mode with the split valve opened after 1.0 min. The injection volume was 1 μl.

Mass spectrometry conditions The mass spectrometry conditions were as follows: ion source temperature 280 ℃, and multiple reaction monitoring (MRM) mode. The retention times and mass spectrometric parameters for various pesticides are presented in Table 1.

Table 1 Mass spectrometry parameters and retention times of 88 pesticides

No.	Pesticide	Retention time//min	Ion pair I	Collision voltage//V	Ion pair II	Collision voltage//V
1	Acephate	4.6	141.0/95.0	5	141.0/79.0	15
2	α-BHC	7.8	218.9/183.0	5	216.9/181.0	5
3	β-BHC	7.9	218.9/183.1	5	216.9/181.1	5
4	β-Endosulfan	12.2	206.9/172.0	15	194.9/124.9	25
5	Aldrin	9.9	262.9/192.9	35	254.9/220.0	20
6	Fenothiocarb	11.0	160.1/106.1	10	160.1/72.1	10
7	Mefenacet	14.8	192.0/136.1	15	192.0/109.1	30
8	Pretilachlor	11.6	262.0/202.2	5	162.1/132.2	20
9	Pyridaben	15.8	147.2/132.2	10	147.2/117.1	20
10	Isoprothiolane	11.5	189.1/145.1	10	162.1/85.0	20
11	Fonofos	8.2	245.9/137.0	5	136.9/109.0	5
12	Dieldrin	11.7	277.0/241.0	5	262.9/193.0	35
13	Dichlorvos	4.7	187.1/93.0	10	184.9/93.0	10
14	Edifenphos	12.9	201.0/173.0	5	201.0/109.0	10
15	Boscalid	16.6	341.9/139.9	15	140.0/112.0	10
16	Chlorpyrifos	10.0	313.8/285.8	5	313.8/257.8	15
17	Parathion	10.0	291.0/81.0	35	290.9/109.0	15
18	Paclobutrazol	11.1	236.0/167.1	10	236.0/125.1	10
19	Diphenylamine	7.0	169.0/168.2	15	168.0/167.2	15
20	Pendimethalin	10.5	251.8/162.2	10	251.8/161.1	15
21	Diazinon	8.3	199.1/135.1	10	199.1/93.0	15
22	Phosalone	14.6	182.0/102.1	15	182.0/75.1	30
23	Fipronil	10.6	368.8/214.8	25	366.8/212.8	25
24	Fipronil sulfoxide	10.6	420.0/350.9	10	351.0/254.9	20
25	Epoxiconazole	13.6	192.0/138.1	10	192.0/111.0	25
26	Cyfluthrin	16.2	198.9/170.1	25	162.9/127.0	5
27	Flucythrinate I	16.7	198.9/107.0	25	156.9/107.1	15
28	Flucythrinate II	16.9	198.9/107.0	25	156.9/107.1	15
29	Flutolanil	11.4	280.9/173.0	10	173.0/95.0	30
30	Procymidone	10.8	284.8/96.0	10	282.8/96.0	10
31	Lambda-cyhalothrin	14.7	208.0/181.0	5	197.0/161.0	5
32	Fludioxonil	11.5	248.0/154.1	20	248.0/127.1	30
33	Hexazinone	13.2	171.0/85.1	10	171.0/71.1	10
34	Hexaconazole	11.4	256.0/82.1	10	231.0/175.0	10
35	Phorate	7.5	260.0/75.0	5	230.9/128.9	25
36	Phorate sulfone	9.9	199.0/143.0	10	199.0/96.9	15
37	Phorate sulfoxide	9.8	199.0/142.9	10	96.9/64.9	20
38	Alachlor	9.3	188.1/160.2	10	188.1/132.1	15
39	Chlorpyrifos-methyl	9.1	287.9/92.9	20	285.9/92.9	20
40	Parathion-methyl	9.1	262.9/109.0	10	232.9/109.0	10
41	Pirimiphos-methyl	9.6	304.9/180.0	5	290.0/125.0	20
42	Fenpropathrin	14.0	264.9/210.0	10	207.9/181.0	5
43	Pirimicarb	8.7	238.0/166.2	10	166.0/96.0	15
44	Quinoxyfen	12.9	306.8/271.9	5	306.8/237.0	20
45	Dimethoate	7.8	86.9/46.0	15	142.9/111.0	10
46	Bifenthrin	13.9	181.2/166.2	10	181.2/165.2	25
47	Phosphamidon	8.8	226.9/127.0	5	192.9/127.0	5
48	Fenarimol	15.1	219.0/107.1	10	219.0/79.0	25
49	Permethrin	15.4	183.1/168.1	10	183.1/153.0	15
50	Cypermethrin	16.5	181.0/152.1	25	163.0/127.0	5
51	Dicloran	7.8	206.1/176	10	160.1/124.1	10
52	Isazofos	8.5	256.9/162.0	5	207.9/165.9	10
53	Malathion	9.7	172.9/99.0	15	157.8/125.0	5
54	Cyprodinil	10.4	225.2/224.3	10	224.2/208.2	20
55	Pyrimethanil	8.3	198.0/158.1	20	198.0/118.1	35
56	Ethoprophos	7	157.9/114.0	5	157.9/97.0	15
57	Mirex	14.8	273.8/238.8	15	271.8/236.8	15
58	Metribuzin	9	198.0/82.0	15	198.0/55.0	30
59	Fenvalerate I	17.4	224.9/119.0	15	167.0/125.1	5
60	Fenvalerate II	17.6	224.9/119.0	15	167.0/125.1	5
61	Tetradifon	14.4	353.8/226.8	10	226.9/199.0	15
62	Triadimenol	10.7	168.0/70.0	10	128.0/100.0	10
63	Triazophos	12.6	161.2/134.2	5	161.2/106.1	10
64	Triadimefon	10	208.0/181.1	5	208.0/111.0	20
65	Tetrachlorvinphos	11.1	332.8/109.0	15	328.9/109.0	15
66	Fenitrothion	9.6	277.0/260.1	5	260.0/125.0	10
67	Methidathion	11	144.9/85.0	5	144.9/58.1	15
68	Isocarbophos	10.1	229.9/212.0	10	229.9/155.0	20
69	cis-Chlordane	11.2	372.9/265.9	20	271.9/236.9	15
70	Terbufos	8.2	230.9/175.0	10	230.9/129.0	20
71	Terbufos sulfone	10.6	198.9/143.0	10	198.9/96.9	20
72	Trifloxystrobin	12.9	116.0/89.0	15	116.0/63.0	30
73	Penconazole	10.5	248.0/192.1	15	248.0/157.1	25
74	Simazine	7.8	201.1/186.2	5	201.1/173.1	5
75	Bromopropylate	13.9	340.8/184.9	20	338.8/182.9	20
76	Deltamethrin	18.1	252.9/174.0	5	250.7/172.0	5
77	Omethoate	6.8	155.9/110.0	5	155.9/79.0	20
78	Acetochlor	9.1	222.9/147.2	5	222.9/132.2	20
79	Ethion	12.4	230.9/175.0	10	230.9/129.0	20
80	Acephate	5.7	136.0/42.0	5	142.0/96.0	5
81	Oxyfluorfen	11.7	299.9/222.8	15	252.0/196.0	20
82	Metolachlor	9.9	238.0/162.2	10	162.2/133.2	15
83	Iprobenfos	8.7	203.9/121.1	35	203.9/91.0	5
84	Coumaphos	15.8	361.9/109.0	15	225.9/163.1	15
85	Ametryn	9.2	227.0/170.1	10	227.0/58.1	10
86	Atrazine	7.9	214.9/200.2	5	214.9/58.1	10
87	Piperonyl butoxide	13.4	176.1/131.1	15	176.1/103.1	25
88	Sulfotep	7.4	321.8/201.9	10	321.8/145.8	25
89	Heptachlor epoxide B (internal standard)	10.6	352.8/316.8	5	352.8/262.9	15

Method validation evaluation

The solvent-diluted standard working solutions prepared in "Sample pretreatment method" were injected for analysis. Standard calibration curves were constructed by plotting the peak area (ordinate) against the mass concentration of the analyte (ng/ml, abscissa). The limit of detection (LOD) for each analyte was determined as the concentration corresponding to a signal-to-noise ratio of three times that of a blank sample. Using carrot as an example matrix, a recovery experiment was conducted at a spiked concentration of 60 ng/ml. The linear ranges, limits of detection, calibration equations, correlation coefficients (R^2), and recovery rates for the 88 pesticides are presented in Table 2. Other matrices were evaluated in the same manner.

Table 2 Linear ranges, limits of detection, recovery rates, calibration equations, and correlation coefficients for 88 pesticides in carrot matrix

No.	Pesticide	Linear range//ng/ml	Limit of detection//μg/kg	Recovery//%	Calibration equation	Correlation coefficient
1	Acephate	10 – 200	0.01	83.1	$y = 582.32x - 4\,671.78$	0.999 5
2	α-BHC	10 – 200	0.01	91.1	$y = 452.53x - 307.41$	0.999 9
3	β-BHC	10 – 200	0.01	87.7	$y = 103.86x - 308.35$	0.999 8
4	β-Endosulfan	10 – 200	0.01	87.9	$y = 49.18x + 174.90$	0.998 9
5	Aldrin	10 – 200	0.01	72.9	$y = 252.71x + 83.98$	0.999 8
6	Fenothiocarb	10 – 200	0.01	79.9	$y = 1\,537.07x - 4\,241.10$	0.999 8
7	Mefenacet	10 – 200	0.01	104.0	$y = 3\,441.83x - 9\,474.56$	0.999 3
8	Pretilachlor	10 – 200	0.01	88.9	$y = 1\,574.05x + 1\,605.55$	0.997 5
9	Pyridaben	10 – 200	0.01	81.2	$y = 5\,338.15x - 10\,492.60$	0.999 8
10	Isoprothiolane	10 – 200	0.01	93.9	$y = 894.79x - 2\,531.38$	0.999 3
11	Fonofos	10 – 200	0.01	95.0	$y = 2\,710.88x - 6\,774.96$	0.999 8
12	Dieldrin	10 – 200	0.01	80.3	$y = 133.56x + 221.38$	0.999 1
13	Dichlorvos	10 – 200	0.01	87.5	$y = 421.96x - 1\,933.26$	0.999 8
14	Edifenphos	10 – 200	0.01	94.4	$y = 626.93x - 2\,302.43$	0.999 7
15	Boscalid	10 – 200	0.01	86.6	$y = 5\,249.09x - 12\,130.40$	0.999 8
16	Chlorpyrifos	10 – 200	0.01	97.1	$y = 360.75x + 893.16$	0.999 5
17	Parathion	10 – 200	0.01	91.8	$y = 332.47x - 1\,942.08$	0.999 9
18	Paclobutrazol	10 – 200	0.01	89.3	$y = 1\,444.29x - 5\,311.35$	0.999 8
19	Diphenylamine	10 – 200	0.01	102.0	$y = 3\,478.06x - 8\,505.33$	0.999 9
20	Pendimethalin	10 – 200	0.01	97.6	$y = 324.69x - 1\,184.14$	0.999 9
21	Diazinon	10 – 200	0.01	96.0	$y = 382.58x - 843.84$	0.999 9
22	Phosalone	10 – 200	0.01	91.3	$y = 1\,334.40x - 8\,645.48$	0.999 6
23	Fipronil	10 – 200	0.01	101.4	$y = 307.40x - 513.08$	0.999 7
24	Fipronil sulfoxide	10 – 200	0.01	95.3	$y = 801.66x + 819.88$	0.999 8
25	Epoxiconazole	10 – 200	0.01	75.7	$y = 1\,883.20x - 5\,366.36$	0.999 6
26	Cyfluthrin	10 – 200	0.01	88.8	$y = 886.21x - 1\,131.98$	0.999 4
27	Flucythrinate I	10 – 200	0.01	92.0	$y = 1\,401.90x - 2\,141.75$	0.999 7
28	Flucythrinate II	10 – 200	0.01	92.0	$y = 1\,193.22x - 2\,187.98$	0.999 8
29	Flutolanil	10 – 200	0.01	95.0	$y = 2\,978.38x - 7\,457.17$	0.999 7
30	Procymidone	10 – 200	0.01	72.9	$y = 487.16x - 254.97$	0.999 6
31	Lambda-cyhalothrin	10 – 200	0.01	80.4	$y = 2\,391.95x - 6\,550.28$	0.999 4
32	Fludioxonil	10 – 200	0.01	89.2	$y = 1\,289.31x - 4\,021.84$	0.999 5
33	Hexazinone	10 – 200	0.01	90.7	$y = 2\,371.34x - 5\,032.82$	0.998 9
34	Hexaconazole	10 – 200	0.01	88.4	$y = 195.98x - 65.61$	0.999 6
35	Phorate	10 – 200	0.01	97.8	$y = 201.22x - 1\,322.69$	0.999 9
36	Phorate sulfone	10 – 200	0.01	93.4	$y = 952.82x - 3\,681.33$	0.999 9
37	Phorate sulfoxide	10 – 200	0.01	91.4	$y = 811.96x - 2\,463.73$	0.999 9
38	Alachlor	10 – 200	0.01	120.0	$y = 863.39x - 171.07$	0.999 9
39	Chlorpyrifos-methyl	10 – 200	0.01	92.6	$y = 482.50x - 1\,081.44$	0.999 9
40	Parathion-methyl	10 – 200	0.01	97.8	$y = 669.83x - 2\,394.30$	0.999 9
41	Pirimiphos-methyl	10 – 200	0.01	95.6	$y = 476.63x - 528.67$	0.999 8
42	Fenpropathrin	10 – 200	0.01	90.8	$y = 439.76x - 377.95$	0.999 2
43	Pirimicarb	10 – 200	0.01	86.3	$y = 930.74x - 1\,679.38$	0.999 6
44	Quinoxyfen	10 – 200	0.01	79.3	$y = 470.86x + 101.44$	0.998 1

(Continued)

(Table 2)

No.	Pesticide	Linear range//ng/ml	Limit of detection//μg/kg	Recovery//%	Calibration equation	Correlation coefficient
45	Dimethoate	10 – 200	0.01	73.1	$y = 873.60x - 4\,521.00$	0.999 9
46	Bifenthrin	10 – 200	0.01	89.3	$y = 7\,082.65x - 12\,662.52$	0.999 2
47	Phosphamidon	10 – 200	0.01	86.1	$y = 106.26x - 644.16$	0.999 7
48	Fenarimol	10 – 200	0.01	96.4	$y = 1\,143.65x - 763.89$	0.999 6
49	Permethrin	10 – 200	0.01	89.7	$y = 932.26x - 545.36$	0.999 2
50	Cypermethrin	10 – 200	0.01	87.5	$y = 995.94x - 3\,274.66$	0.999 6
51	Dicloran	10 – 200	0.01	72.3	$y = 453.85x - 942.62$	0.999 6
52	Isazofos	10 – 200	0.01	94.2	$y = 199.20x - 413.11$	0.999 8
53	Malathion	10 – 200	0.01	92.4	$y = 1\,376.70x - 3\,171.91$	0.999 8
54	Cyprodinil	10 – 200	0.01	74.9	$y = 2\,860.72x - 4\,791.93$	0.999 8
55	Pyrimethanil	10 – 200	0.01	84.8	$y = 608.60x - 722.28$	0.999 7
56	Ethoprophos	10 – 200	0.01	82.7	$y = 1\,022.40x - 2\,271.85$	0.999 8
57	Mirex	10 – 200	0.01	79.9	$y = 1\,326.43x - 2\,259.47$	0.999 8
58	Metribuzin	10 – 200	0.01	84.9	$y = 538.50x - 1\,685.58$	0.999 9
59	Fenvalerate I	10 – 200	0.01	94.4	$y = 1\,165.16x - 1\,746.79$	0.999 6
60	Fenvalerate II	10 – 200	0.01	90.6	$y = 515.54x - 1\,222.64$	0.999 5
61	Tetradifon	10 – 200	0.01	77.9	$y = 487.67x - 1\,141.98$	0.999 8
62	Triadimenol	10 – 200	0.01	90.1	$y = 776.28x - 2\,075.12$	0.999 7
63	Triazophos	10 – 200	0.01	93.6	$y = 756.79x - 6\,950.41$	0.999 6
64	Triadimefon	10 – 200	0.01	87.1	$y = 659.63x + 286.08$	0.999 8
65	Tetrachlorvinphos	10 – 200	0.01	97.9	$y = 715.11x - 3\,499.98$	0.999 6
66	Fenitrothion	10 – 200	0.01	96.8	$y = 496.76x - 1\,536.13$	0.999 7
67	Methidathion	10 – 200	0.01	89.8	$y = 3\,209.73x - 22\,238.12$	0.999 9
68	Isocarbophos	10 – 200	0.01	93.2	$y = 178.07x - 1\,377.30$	0.999 9
69	cis – Chlordane	10 – 200	0.01	81.3	$y = 205.61x - 69.10$	0.999 6
70	Terbufos	10 – 200	0.01	97.2	$y = 1\,076.03x - 7\,460.05$	0.999 8
71	Terbufos sulfone	10 – 200	0.01	93.6	$y = 621.73x - 1\,034.75$	0.999 9
72	Trifloxystrobin	10 – 200	0.01	96.1	$y = 2\,184.11x - 3\,377.55$	0.999 8
73	Penconazole	10 – 200	0.01	92.8	$y = 1\,057.96x - 1\,000.13$	0.999 8
74	Simazine	10 – 200	0.01	79.4	$y = 285.21x - 777.96$	0.999 9
75	Bromopropylate	10 – 200	0.01	95.3	$y = 980.66x - 374.43$	0.999 9
76	Deltamethrin	10 – 200	0.01	104.6	$y = 228.02x - 310.94$	0.999 9
77	Omethoate	10 – 200	0.01	80.3	$y = 321.45x - 3\,787.18$	0.998 8
78	Acetochlor	10 – 200	0.01	101.7	$y = 447.74x + 256.69$	0.999 9
79	Ethion	10 – 200	0.01	97.0	$y = 1\,712.35x - 7\,248.29$	0.999 5
80	Acephate	10 – 200	0.01	72.3	$y = 380.32x - 3\,682.22$	0.999 5
81	Oxyfluorfen	10 – 200	0.01	80.5	$y = 178.45x - 468.70$	0.999 2
82	Metolachlor	10 – 200	0.01	100.1	$y = 2\,634.60x - 3\,345.05$	0.999 8
83	Iprobenfos	10 – 200	0.01	97.4	$y = 190.30x - 711.50$	0.999 9
84	Coumaphos	10 – 200	0.01	81.1	$y = 264.48x - 983.14$	0.999 6
85	Ametryn	10 – 200	0.01	86.1	$y = 371.97x + 924.16$	0.999 7
86	Atrazine	10 – 200	0.01	91.9	$y = 290.22x - 448.61$	0.999 9
87	Piperonyl butoxide	10 – 200	0.01	96.7	$y = 2\,805.06x - 6\,892.04$	0.999 4
88	Sulfotep	10 – 200	0.01	94.6	$y = 275.02x - 214.70$	0.999 8

As shown in Table 2, all 88 pesticides exhibited good linear relationships within the concentration range of 10 – 200 ng/ml, with correlation coefficients (R^2) all greater than 0.99 and stable slopes of the calibration equations. These results indicate that the method has excellent quantitative accuracy and reliability over a wide dynamic range. The limits of quantification (LOQ) and sensitivity of the method meet the requirements for the detection of trace pesticide residues. Recovery validation performed using the carrot matrix demonstrated that at a spiked level of 60 ng/ml, the average recovery rates of the 88 pesticides ranged from 72.3% to 120.0%, with relative standard deviations (*RSD*, $n = 6$) ranging from 5.3% to 15.2%. The precision and accuracy of the method were both in compliance with the technical requirements specified in GB/T 27404-2026 *Criterion on Quality Control of Laboratories—Chemical Testing of Food*, which stipulate that for spiked levels greater than 10 μg/kg and up to 100 μg/kg, the recovery

should be between 70% and 120%, and the *RSD* should be within the range of −30% to +20%. Further evaluation of the recovery performance at two additional spiked levels, a low level (20 ng/ml) and a high level (120 ng/ml), was conducted. The results showed that at the three spiked levels, the recovery rates at the low concentration level exhibited slight fluctuations, but remained within acceptable ranges, while the recovery stability at the medium and high concentration levels was significantly improved. These findings confirm that the method has good applicability and robustness at different residue levels, thereby providing reliable methodological support for the subsequent systematic evaluation of matrix effects from multiple matrices.

Evaluation Method for Matrix Effects

Matrixmatched standard solutions were prepared at the same concentration levels using blank sample extracts, with five replicates for each matrix. The matrix effect (ME) was evaluated by comparing the relative peak area ratios of the target compounds obtained from the matrixmatched standard solutions with those obtained from the solvent standard solutions. The matrix effect for each sample was calculated according to the following formula[15]:

$$ME = \frac{B}{A} \times 100\%$$

In the formula, A represents the peak area of the target compound in the solvent standard solution, and B represents the corresponding peak area in the matrixmatched standard solution. For each matrix, the five replicate data sets were processed by removing the maximum and minimum values, and the remaining three values were averaged.

When the *ME* value exceeds 100%, a matrix enhancement effect is indicated. Conversely, when the *ME* value is below 100%, a matrix suppression effect is indicated. According to the intensity of the matrix effect, classification was conducted according to specific criteria. Specifically, if $|ME - 100\%| \leqslant 20\%$ (*i. e.*, $80\% \leqslant ME \leqslant 120\%$), the matrix effect is considered weak. Among these, $80\% \leqslant ME < 100\%$ indicates weak matrix suppression, and $100\% < ME \leqslant 120\%$ indicates weak matrix enhancement. If $20\% < |ME - 100\%| \leqslant 50\%$ (*i. e.*, $50\% \leqslant ME < 80\%$ or $120\% < ME \leqslant 150\%$), the matrix effect is considered medium. Among these, $50\% \leqslant ME < 80\%$ indicates medium matrix suppression, and $120\% < ME \leqslant 150\%$ indicates medium matrix enhancement. And if $|ME - 100\%| > 50\%$ (*i. e.*, $ME < 50\%$ or $ME > 150\%$), the matrix effect is considered strong. Among these, $ME < 50\%$ indicates strong matrix suppression, and $ME > 150\%$ indicates strong matrix enhancement.

According to standards such as GB 23200.113 and GB 23200.8, when GC-MS is used to determine pesticide residues in fruits and vegetables, significant differences in matrix effects were observed among different fruit and vegetable matrices for various pesticide components. This is one of the main reasons for deviations in quantitative pesticide residue analysis. Based on this, the present study aims to minimize quantitative detection errors and improve the efficiency of batch sample screening. To provide a technical reference for the practical application of multipesticide residue detection in fruits and vegetables and for related scientific research, this study innovatively proposes the construction of a matrix effect (*ME*) classification system for multipesticide residue detection by GCMS/MS, using a gradient interval of 10% for the matrix effect. Based on the experimental data, the matrix effect was divided into a total of 16 gradient intervals: 50% −60%, 60% −70%, 70% −80%, 80% −90%, 90% −100%, 100% −110%, 110% −120%, 120% −130%, 130% −140%, 140% −150%, 150% −160%, 160% −170%, 170% −180%, 180% −190%, 190% −200%, and >200%. These intervals are intended for the comprehensive evaluation of the matrix effects of different fruit and vegetable matrixpesticide combinations.

Overall evaluation of matrix effects

Overall, the matrix effect data for the 88 pesticides in the 11 vegetables and 9 fruits showed a highly concentrated distribution, with matrix enhancement being the dominant effect, accounting for 95.8% of the total. More than 87% of the data points were concentrated in the 120% −150% and >150% intervals, and high residual levels were common. The matrix effect values for most fruit and vegetable samples were above 100%. Among these, a medium matrix enhancement effect (*ME* value of 120% −150%) was the most typical, accounting for 54.9%, followed by a relatively strong matrix enhancement effect (*ME* value >150%), accounting for 32.3%. A weak matrix enhancement effect (*ME* value of 100% −120%) accounted for a relatively small proportion, 8.6%. Matrix suppression effects accounted for a relatively small proportion, totaling 4.2%, and no weak matrix suppression effect with an *ME* value <50% was observed, as specifically shown in Fig. 1 below.

After analyzing the matrix effects of 88 pesticides in 11 vegetables and 8 fruits (Table 3), from the matrix perspective, the matrix effect intervals of 130% −140% and 140% −150% were the most typical, each accounting for more than 20% of the total. These intervals covered all matrix types, with typical matrices represented by sweet orange, tangerine, cucumber, ginger, and mango. Considering the influence of matrix components on the matrix effect, sweet orange and tangerine (containing sugars, pectin, waxes, pigments, and organic acids), ginger (containing gingerols, volatile oils/terpenes, and fiber), and mango (containing extremely high sugar content, high organic acids, and carotenoids) all contain abundant high-concentration interfering substances. In contrast, cucumber has a relatively simple matrix composition. It has high water content, low soluble sugar content, and very low endogenous secondary metabolite content, with almost no strongly interfering polyphenols, tannins, or essential oils. Therefore, cucumber is recommended as the most typical representative matrix. The matrix effect intervals of 120% −130% and 150% −160% were considered as representative of sub-typical intervals, each accounting for more than 14% of the total matrix effect and covering all matrix types, with typical matrices represented by bean sprout, carrot, and ginger. Among all matrix effect intervals, the matrix suppression effect was most pronounced in sugar orange, followed by romaine lettuce, Chinese chive, and scallion. This may be related to the dual mechanisms of charge competition

and co-elution inhibition caused by the high sugar, high acid, high polyphenol/flavonoid, and high wax/terpene contents in sugar orange. Romaine lettuce has high contents of chlorophyll pigments, waxy lipids, and polyphenolic salts, with pigments and surface substances dominating the suppression effect. Chinese chive and scallion contain high levels of sulfur-containing volatile compounds, and the coelution of chlorophyll, waxes, and lipids is severe. Moreover, these matrices have a wide variety and high concentrations of endogenous secondary metabolites, which further amplify the suppression effect. These observations indicate that the pesticide matrix effect is closely related to the matrix components inherently present in the samples, such as plant secondary metabolites, pigments, organic acids, sugars, pectin, and waxes.

From the perspective of pesticide type distribution, the intervals of 130% – 140% and 140% – 150% were the dominant matrix effect intervals, encompassing more than 86% of the tested pesticides, and phorate sulfoxide, diazinon, and pyridaben were representative examples. These compounds have similar molecular weights and polarities. Their molecular structures often contain sulfur, phosphorus, nitrogen heterocycles, chlorine substituents, and ether/thioether bonds, and they belong to groups such as organophosphorus, heterocyclic, amide, and triazole pesticides. Their ionization tendencies are relatively consistent, and therefore their matrix effect characteristics are similar. The intervals of 120% – 130% and 150% – 160% were the subdominant intervals, with pesticide coverage reaching 80%, and the representative examples included dichlorvos, malathion, fenothiocarb, and diphenylamine. These compounds have similar physicochemical properties and boiling point ranges, all possessing a hydrophobic carbon skeleton with O, S, and N heteroatom substitutions. Their structures and adsorption characteristics are similar, and therefore, their matrix effect patterns are consistent. Matrix suppression effects were mainly observed for edifenphos, tetrachlorvinphos, deltamethrin, fenvalerate I, and lambdacyhalothrin in matrices such as romaine lettuce, Shanghai bok choy, and celery. These pesticide compounds have high molecular weights and boiling points, and low volatility. Their vaporization at high temperatures is incomplete. In addition, they contain benzene rings, halogen atoms, ester bonds, and sulfur/phosphorus structures, making them prone to adsorption at active sites. They also exhibit poor thermal stability, strong retention, and late elution. Consequently, they are more significantly affected by interference from co-extracted matrix components, leading to pronounced matrix suppression. Paclobutrazol, triazophos, phorate, dicloran, terbufos, and fenothiocarb consistently exhibited stable and relatively strong matrix enhancement effects in all 19 fruit and vegetable matrices. This behavior is mainly attributed to their medium-low boiling points, relatively weak polarity, and the presence of multiple polar functional groups, which make them prone to adsorption at active sites in the GC inlet and on the chromatographic column. Meanwhile, matrix components such as waxes and long-chain fatty acids in fruits and vegetables have boiling points similar to those of these pesticides, thereby reducing their adsorption and retention, and consequently leading to pronounced matrix enhancement effects.

In summary, the matrix effect of pesticides is not determined solely by the matrix type, but is also closely related to their chemical structure, polarity, chromatographic retention behavior, and ionization efficiency. Therefore, when quantifying multiple pesticides simultaneously, classification analysis and differentiated correction must be performed according to both pesticide type and matrix type, in order to ensure the quantitative accuracy of multi-residue detection.

Table 3 Statistical table of matrix effect analysis across different intervals

No.	Matrix effect intensity	Proportion %	Vegetable	Compound	Typical matrix	Typical pesticide
1	0% – 50% matrix effect	0.0	0	0	None	None
2	50% – 60% matrix effect	0.2	3	2	Carrot, celery, and Chinese chive	Edifenphos and tetrachlorvinphos
3	60% – 70% matrix effect	0.3	4	2	Chinese chive (first choice), followed by scallion, romaine lettuce, and sweet orange	Edifenphos and tetrachlorvinphos
4	70% – 80% matrix effect	1.3	11	4	Sugar mandarin and Shanghai bok choy	Deltamethrin
5	80% – 90% matrix effect	1.1	13	6	Sugar mandarin	Deltamethrin
6	90% – 100% matrix effect	1.6	15	10	Sugar mandarin and romaine lettuce	Fenvalerate I
7	100% – 110% matrix effect	3.1	19	19	Celery, tangerine, and sugar mandarin	Lambda-cyhalothrin and fenvalerate I
8	110% – 120% matrix effect	6.3	19	32	Bean sprout	Fenitrothion
9	120% – 130% matrix effect	15.6	19	68	Bean sprout	Dichlorvos and malathion
10	130% – 140% matrix effect	21.9	19	76	Sweet orange, tangerine, and cucumber	Phorate sulfoxide, diazinon, pyridaben, pirimiphos-methyl, and alachlor
11	140% – 150% matrix effect	20.5	19	77	Ginger and mango	Cyprodinil, hexaconazole, and sulfotep
12	150% – 160% matrix effect	14.4	19	71	Carrot and ginger	Fenothiocarb and diphenylamine
13	160% – 170% matrix effect	10.8	19	63	Cowpea, pepper, and carrot	Paclobutrazol
14	170% – 180% matrix effect	5.0	17	38	Pepper (first choice), followed by Chinese chive	Triazophos and terbufos
15	180% – 190% matrix effect	2.2	11	24	Chinese chive	Dicloran
16	190% – 200% matrix effect	1.0	8	12	Chinese chive (first choice), followed by pepper	Dicloran
17	>200% matrix effect	1.0	9	8	Ginger (first choice), followed by Chinese chive	Acephate

Matrix effect analysis of the same pesticide in different matrices

The matrix effect is a major interference factor in the analysis of pesticides in complex samples, directly affecting quantitative accuracy and method reliability. In this study, the response patterns of 88 pesticides in 19 fruit and vegetable matrices were investigated, and it was found that the matrix effect exhibited significant dual dependence on both the compound and the matrix. As shown in Fig. 2, the most predominant matrix effect was the medium matrix enhancement effect of 120% – 150%, accounting for 54.9% of the total. The relatively strong matrix enhancement effect (>150%) ranked second, accounting for 32.3%, while the weak matrix enhancement effect (100% – 120%) accounted for only 8.6%. The proportion of matrix suppression effects was extremely low. Weak suppression effects in the range of 80% – 100% were observed for only 12 pesticides, including deltamethrin and tetrachlorvinphos, accounting for 2.6% of the total. Relatively strong suppression effects in the range of 50% – 80% were observed for only four pesticides, namely, tetrachlorvinphos, edifenphos, deltamethrin, and fenvalerate I, accounting for 1.6% of the total. The occurrence of matrix suppression effects for these pesticides can be mainly attributed to the inherent polarity, structural functional groups, and thermal stability characteristics of pyrethroids, organophosphorus pesticides, and metribuzin. At the high temperature of the injection port, these compounds are prone to pyrolysis and catalytic degradation, and they undergo competitive adsorption with coextracted matrix components, collectively inducing a matrix suppression effect.

Further analysis of the matrix effects in all matrices revealed that six compounds exhibited a relatively strong matrix enhancement effect (>150%) in every matrix tested. Thirteen compounds consistently showed either weak (100% – 120%) or medium (120% – 150%) matrix enhancement effects in all 19 matrices. In contrast, six compounds displayed a unique matrix suppression effect in certain matrices. The similar patterns of signal enhancement or suppression observed for these compounds in multiple matrices can be mainly attributed to the combined effects of their core functional groups and coextracted matrix components (such as pigments, waxes, sulfurcontaining substances, water content, and secondary metabolites).

Analysis of similar types of matrix effects Among the 88 pesticides, some compounds exhibited similar levels of matrix effects across different matrices, demonstrating good matrix stability. Specific results are shown in Fig. 3 and Fig. 4. The differences in the matrix effects of these pesticides arise from common interferences inherent to the matrices themselves on the one hand, and from differences in specific components of the matrices on the other hand, which also influence the expression of their matrix effects.

As shown in Fig. 3, six compounds, paclobutrazol, terbufos, fenothiocarb, triazophos, phorate, and dicloran, exhibited a relatively strong matrix enhancement effect (*ME* values ranging from 150% to 220%) in 18 of the matrices except bean sprouts. Even in the bean sprout matrix, the matrix effect values for these pesticides were close to 150%, with a mean value of 145%, as specifically shown in Fig. 3. As can be seen from the figure, the matrix effect values for the abovementioned compounds were highest in pepper and Chinese chive (mean *ME* value 200%), and the matrix effects in vegetables were slightly higher than those in fruits. In the eight fruits, the *ME* values of the matrix effect remained stable within the range of 160% ± 10%. It may be related to the low water content, high sulfur content (in Chinese chive), high pigment/wax content (in pepper), and high polarity organic matter in pepper and Chinese chive, whereas tropical and citrus fruits have high water content, fewer interfering substances, and relatively simpler matrices. Consequently, stronger matrix enhancement effects were observed in the detection of pepper and Chinese chive. In summary, during routine analysis, the six pesticides mentioned above should be quantified using matrixmatched calibration curves specific to pepper and Chinese chive. If solvent calibration curves are used for quantitation, attention should be paid to applying a matrix effect correction factor ($MF = 1/ME$) for conversion to eliminate systematic bias. For fruits, more readily available matrices such as tangerine or sugar mandarin may be considered as universal matrices for quantitation.

As shown in Fig. 4, a total of 13 compounds, namely, dichlorvos, α-BHC, β-BHC, chlorpyrifos-methyl, parathion-methyl, fenitrothion, malathion, fipronil, cyfluthrin, cypermethrin, boscalid, flucythrinate I, and flucythrinate II, exhibited mainly weak to medium matrix enhancement effects (*ME* values of 100% – 150%) in the 19 matrices. In Chinese chive (for fipronil) and ginger (for chlorpyrifos-methyl, parathion-methyl, and fenitrothion), the matrix effect values were >150%, indicating a relatively strong matrix enhancement effect. Among them, dichlorvos, α-BHC, and chlorpyrifos-methyl exhibited a medium matrix enhancement effect in various fruits ($ME = 127\% \pm 8\%$, $CV = 3.1\%$). β-BHC and malathion showed a similar enhancement effect in all fruits ($ME = 122\% \pm 6\%$, $CV = 4.2\%$). Parathion-methyl and fenitrothion showed a slightly weaker matrix enhancement effect in various fruits ($ME = 116\% \pm 9\%$, $CV = 5.8\%$). These results indicate that the matrix effects of these pesticides are generally stable in the above-mentioned tropical and citrus fruits. Compared with fruit matrices, the matrix effects of the pesticides varied more widely in vegetable matrices. Stronger matrix effects were observed in ginger, Chinese chive, pepper, cowpea, carrot, and scallion, while weaker matrix effects were observed in bean sprout, cucumber, Shanghai bok choy, and celery. It may be attributed to the fact that ginger, Chinese chive, pepper, and other vegetables have a high sulfur content, high pigment content, high wax content, low water content, and complex secondary metabolites, leading to strong matrix interference. In contrast, bean sprout, cucumber, Shanghai bok choy, and celery have high water content, low sulfur content, low pigment content, thin wax layers, and simple endogenous components, resulting in weaker matrix effects. After comprehensive consideration, for the quantification of the above 13 compounds, dragon fruit may be considered as a u-

niversal matrix for tropical fruits in fruit matrices, while tangerine may be considered as a universal matrix for citrus fruits. As to vegetable matrices, for relatively weak matrix enhancement effects, cucumber and bean sprout may be used as universal matrices, and for medium matrix enhancement effects, pepper and cowpea may be used as universal matrices.

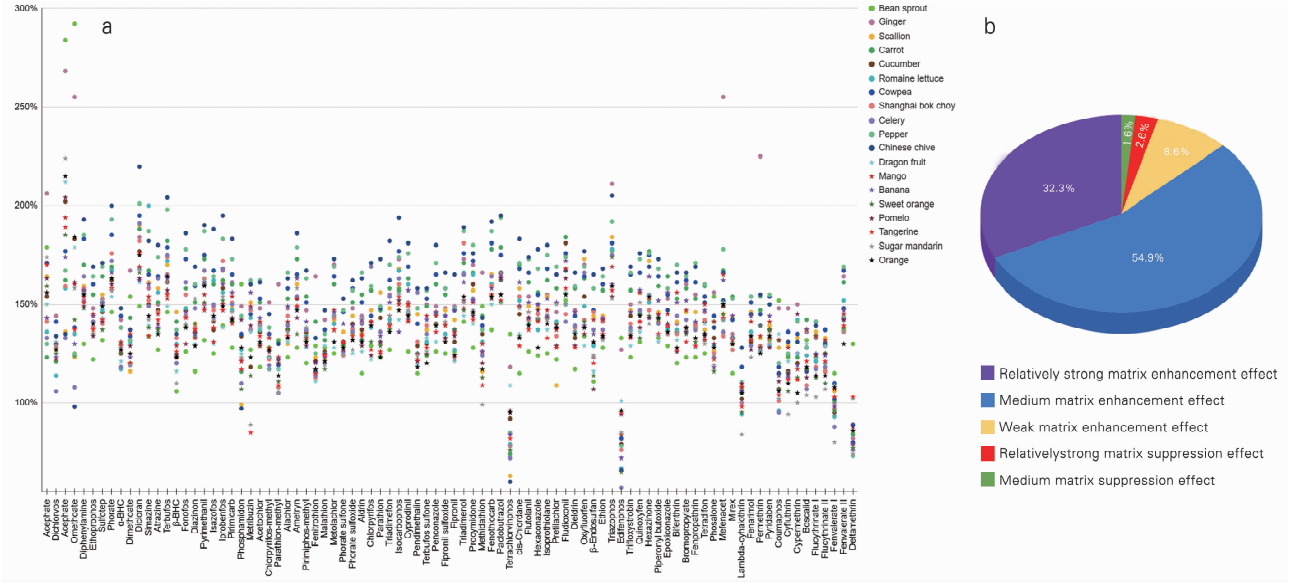


Fig. 1 Comprehensive map of matrix effects of 88 pesticides (a) and matrix effect proportion diagram for each interval range (b)

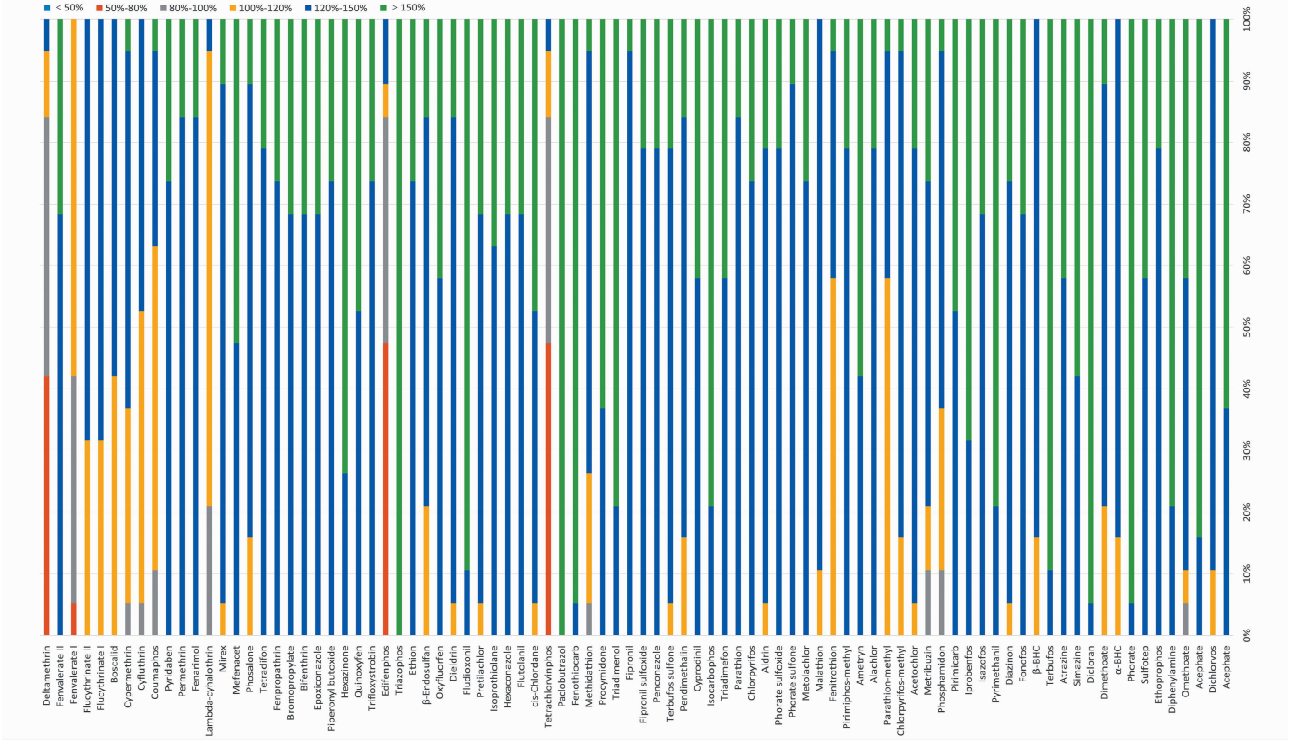


Fig. 2 Distribution of different matrix effects among 88 pesticides in 19 fruits and vegetables

Analysis of special types of matrix effects GS-MS detection of pesticide residues is dominated by matrix enhancement effects. However, some pesticides may exhibit matrix suppression effects due to their own physicochemical properties and interference from co-extracted matrix components. As shown in Fig. 5 (numbers 1 – 19 represent 19 matrices including bean sprout, ginger and scallion), deltamethrin, tetrachlorvinphos, edifenphos, fenvalerate I, lambda-cyhalothrin, and metribuzin all exhibited clear matrix suppression effects. Among these, deltamethrin, tetrachlorvinphos, and edifenphos exhibited suppression effects in all matrices except bean sprout and dragon fruit, and medium suppression levels (ME values below 80%) were observed in many cases. For

tetrachlorvinphos, the *ME* value was as low as 62% in scallion and Chinese chive, and it averaged approximately 76% in various other vegetables and citrus fruits. For edifenphos, the ME value in carrot and celery was 57%, and the mean values of 66% and 76% were exhibited in other different matrices, respectively. For deltamethrin, the mean *ME* value in most vegetables, banana, and sweet orange was 77%. For the special items in the above special matrices, those with similar matrix effect levels may share a common matrix for quantitation. However, for different groups of samples, dedicated matrix-matched calibrations should be established. If solvent calibration curves are used for initial screening quantitation, matrix effect correction factors must be applied for adjustment and compensation, in order to obtain accurate and reliable quantitative results.

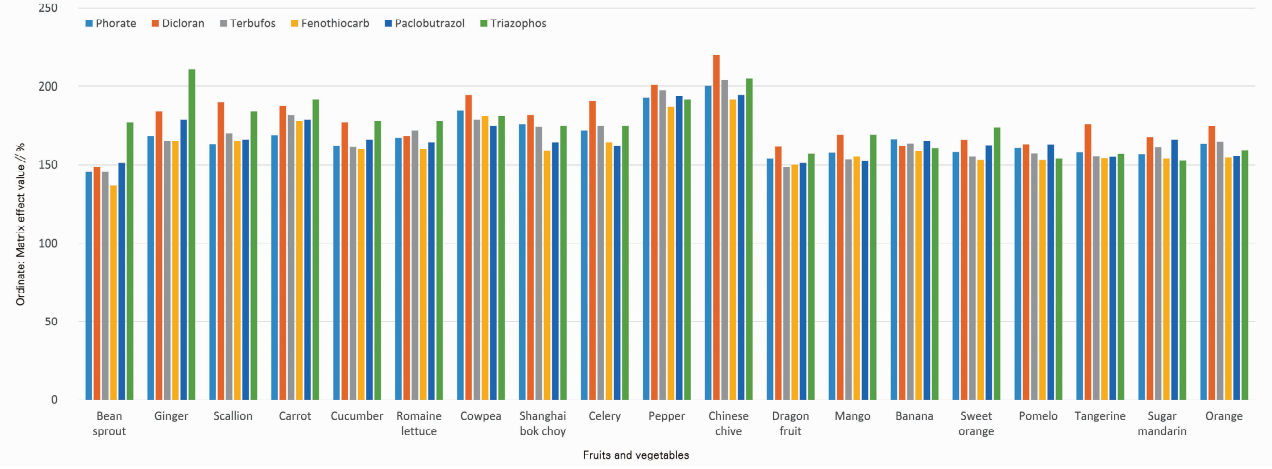


Fig. 3 Relativley strong matrix enhancement effect diagram of six pesticides

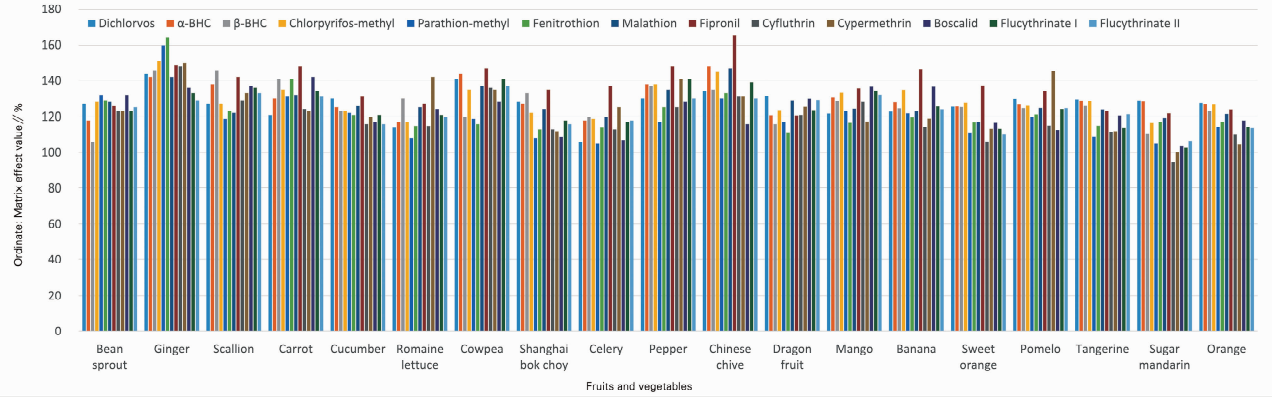


Fig. 4 Weak and medium matrix enhancement effects of 13 pesticides

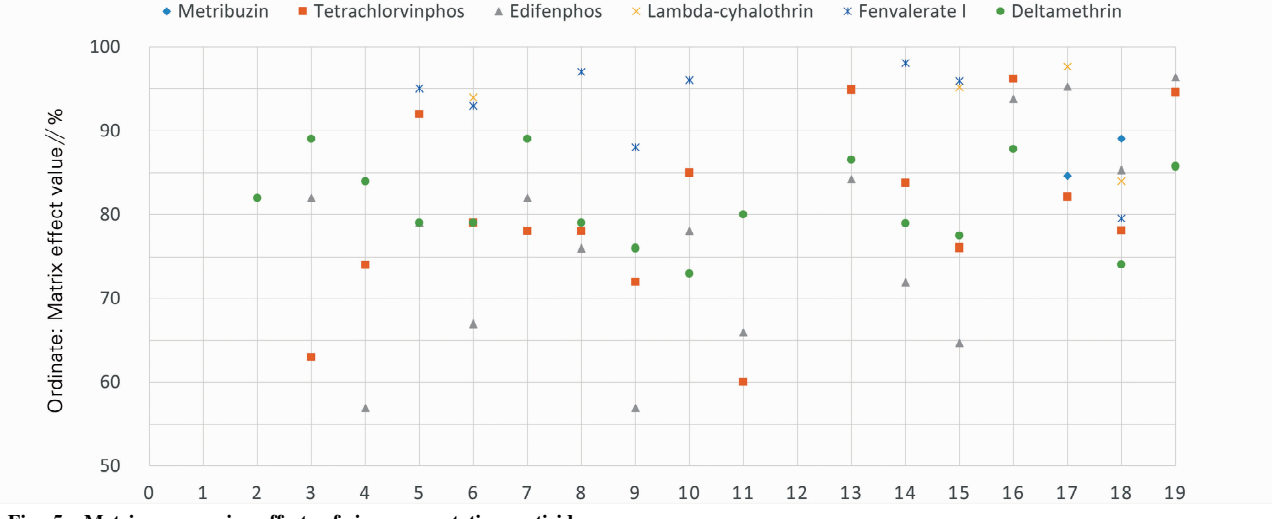


Fig. 5 Matrix suppression effects of six representative pesticides

Matrix effect analysis of different pesticides in the same matrix

In the same type of matrix environment, different pesticides exhibit significant compound-specific differences in their matrix effects. Taking typical fruit and vegetable matrices such as pepper and sugar orange as examples, polar pesticides such as organophosphorus compounds are often interfered with by co-extracted substances such as sugars and organic acids in the matrix, leading to signal enhancement. In contrast, non-polar pesticides such as pyrethroids are more susceptible to suppression by lipophilic substances such as chlorophyll and waxes, resulting in a decrease in detection response. This difference in matrix effect is mainly caused by the competition with co-extracted matrix components at the column head, ion source, or detector due to the physicochemical properties of the pesticides themselves (including polarity, functional group types, and thermal stability). Studies have found that the matrix effects of isomeric pesticides (such as fenvalerate I and fenvalerate II) may differ considerably. This phenomenon indicates that even slight differences in the molecular structure of a pesticide can alter the interference characteristics exerted by the matrix. Based on this, when establishing multi-residue detection methods, it is necessary to perform group-specific optimization according to the inherent characteristics of the target pesticides. By adopting strategies such as matrix-matched calibration or isotope internal standard correction, the quantitative deviations caused by compound-specific matrix effects can be effectively eliminated, thereby ensuring the accuracy and reliability of simultaneous detection of multiple pesticides in complex samples.

Overall discussion of matrix effects in the same matrix To comprehensively evaluate matrix universality, this study analyzed the distribution characteristics of the matrix effects of 88 pesticides in 11 vegetable matrices and 8 fruit matrices. The results are shown in Fig. 6. According to the intensity of the matrix effect, the effects were classified into three categories: weak matrix effects ($80\% \leqslant ME \leqslant 120\%$), medium matrix effects ($50\% \leqslant ME < 80\%$ or $120\% < ME \leqslant 150\%$), and relatively strong matrix effects ($ME < 50\%$ or $ME > 150\%$). Since the proportion of medium matrix effects was generally higher than that of other two categories in all matrices, the proportion of medium matrix effects was used as the core evaluation indicator for matrix universality.

The analysis results showed that the proportions of medium matrix effects in eight matrices, namely, bean sprout, dragon fruit, pomelo, cucumber, Shanghai bok choy, mango, sweet orange, and tangerine, were significantly higher than those in other matrices, indicating that these eight matrices exhibit excellent matrix universality characteristics. Among these, pomelo, dragon fruit, and bean sprout exhibited the most optimal distribution of matrix effects. For pomelo and dragon fruit, the proportion of medium matrix effects reached 76.1%, while the combined proportion of weak and strong matrix effects was only 23.8% and 23.9%, respectively. For bean sprout, the proportion of medium matrix effects was 71.6%, and the combined proportion of weak and relatively strong matrix effects was 28.4%. For all three matrices, the proportions of relativley weak and relativley strong extreme matrix effects were the lowest among the 19 matrices, which is consistent with the principles for selecting universal matrices. Moreover, bean sprout and dragon fruit showed no matrix suppression effects, and their matrix effect profiles are relatively simple. Therefore, they can be recommended as preferred representative matrices for crosscategory multi-pesticide residue detection.

In contrast, for five matrices, pepper, Chinese chive, carrot, cowpea, and ginger, relativley strong matrix effects were dominant. Except for ginger where the proportion of relatively strong matrix effects was slightly below 60%, the proportion exceeded 60% for all the others, and weak matrix effects accounted for the smallest proportion in this group of matrices, none exceeding 8%. Therefore, considering the specificity of matrix effects in different categories, this class of matrices with strong matrix effects must be quantified separately from the "universal matrices". The indiscriminate use of a common matrix would introduce significant systematic bias and reduce the accuracy of simultaneous multi-pesticide residue detection. Of course, for the above five matrices, except for ginger, the other four all exhibited medium matrix suppression effects with ME values in the range of 50% – 80%. Taking into account the specific components of each matrix (ginger and Chinese chive contain large amounts of sulfur-containing compounds, gingerols, and other highly volatile, strongly interfering substances) and the proportions of matrix suppression effects, cowpea is recommended as a universal matrix for this type of strong matrix effect.

Discussion of weak matrix effects Based on the statistical results of matrix effect grouping, this study further identified pesticide-matrix combinations that exhibited weak matrix effects. Among the 19 matrices, a total of 36 pesticides, including fenvalerate I, lambda-cyhalothrin, coumaphos, parathion-methyl, and fenitrothion, exhibited matrix suppression effects. These effects were most pronounced in celery and sugar orange, followed by orange and sweet orange. The specific distribution patterns are shown in Fig. 7. As can be seen from the figure, the matrix effect data for bean sprout, Shanghai bok choy, dragon fruit, and sweet orange were relatively stable, with the smallest fluctuations and an average ME value of 110%. Considering the influence of matrix component complexity on the matrix effect, bean sprout is recommended as a universal calibration matrix. For ginger, cowpea, and pepper, although some data showed weak matrix suppression effects, the values fluctuated considerably. It is recommended that, based on actual testing needs, specific analytes should be calibrated with matrixmatched standard curves for corresponding matrices to reduce quantitative bias.

Discussion of strong matrix effects In addition to the typical medium matrix effects and the special weak matrix effects, relatively strong matrix effects (referring in this study to relatively strong matrix enhancement effects) were also very pronounced in the different matrices, as specifically shown in Fig. 8 below. This interval involved 19 matrices, 74 pesticides, and 523 matrix effect data points, accounting for 31.3% of the entire matrix effect range. As can be seen from the figure, most matrix effects were concentrated in the ME value range of 150% – 180%. For some analytes, such as triazophos, triadimenol, and acephate, the matrix effects fluctuated considerably among the different matrices. Six analytes, namely, triazophos, acephate, omethoate, dicloran,

permethrin, and mefenacet, even exhibited matrix enhancement effects greater than 200%. This may be related to the fact that the abovementioned compounds belong to substance classes with high polarity and a strong tendency to form hydrogen bonds, including organophosphorus compounds (triazophos, acephate, omethoate, isocarbophos, phorate, *etc.*) whose structures commonly contain $P=O/P=S$, triazole rings, amide/amino groups, ureido groups, cyano groups, or imidazole/pyridine rings, triazoles (triadimenol, paclobutrazol), and carbamates/ureas (pirimicarb, ametryn, simazine). Among all the matrices, pepper, Chinese chive, carrot, and cowpea exhibited the most significant strong matrix enhancement effects, which is related to their complex and special matrix components. To ensure efficiency while achieving more accurate quantitation in multianalyte detection, it is recommended that for relatively strong matrix enhancement effects of the same category, a universal matrix such as pepper or cowpea be used for quantitation. For analytes with specific enhancement effects, typical matrices in the corresponding interval (Table 3) should be selected for correction. If solvent calibration curves are used for calibration, attention must be paid to applying the matrix effect correction factor ($MF=1/ME$) for conversion before determining the final values.

Discussion of matrix effects of different pesticides within the same interval Based on the systematic evaluation of the matrix effects of 88 pesticides in 19 fruit and vegetable matrices, the matrix effects were divided into five characteristic intervals according to their intensity. The response patterns of pesticides and matrices within each interval were analyzed to provide a basis for establishing differentiated quantitation strategies.

120% – 130% (relatively weak enhancement): The pesticides in this interval were represented by dichlorvos and malathion. These compounds have relatively weak polarity, large steric hindrance, and good thermal stability, and therefore only produce a weak matrix enhancement effect. This interval accounted for 15.6% of the total. To improve detection efficiency while reducing quantitative bias, bean sprout matrix is recommended for calibration standard preparation.

130% – 140% (weak enhancement): Covering 76 pesticides, this interval accounted for the largest proportion and represented the typical distribution region of matrix effects. Among these, 18 pesticides, such as pendimethalin, diphenylamine, and phosalone, exhibited significant matrix specificity, with only one or two matrices falling within this interval for each pesticide, and for the remaining matrices, the ME values showed a wide dispersion. These pesticides have a broad range of structures and polarities and are sensitive to environmental factors such as matrix pH, ionic strength, and coextracted components. Therefore, attention should be paid to a "one pesticide, one matrix" approach, and blank matrix calibration should be adopted for standard preparation.

140% – 150% (medium enhancement): It was the second major enhancement interval and contained the largest number of pesticide types. Fifteen pesticides, including dimethoate, boscalid, and dichlorvos, showed strong matrix dependence, with only a few matrices falling within this interval and ME values exhibiting wide fluctuations. Quantitative detection using solvent-prepared calibration standards would introduce significant systematic error, and therefore matrixmatched calibration or isotope internal standard correction must be conducted.

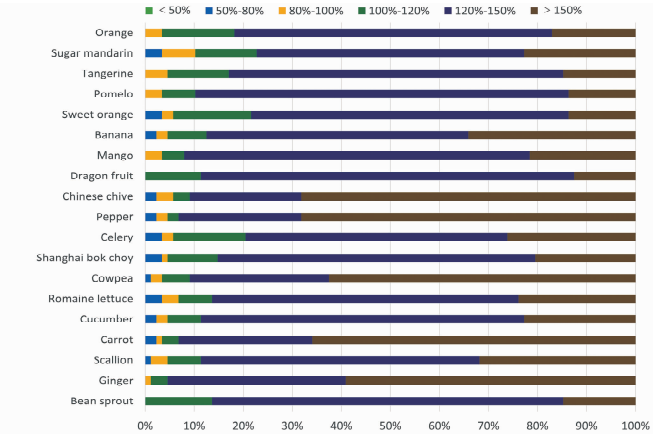
150% – 160% (relatively strong enhancement): This interval involved 71 pesticides, and covered all 19 matrices, with carrot and ginger being the most typical matrices. The strongest effects were observed for fenothiocarb and diphenylamine. These compounds contain strongly polar functional groups and aromatic skeletons, which synergistically reduce the loss of analytes in the presence of the matrix, resulting in a pronounced matrix enhancement effect. For these two compounds, a cross-matrix universal calibration curve can be established, using carrot as the representative matrix for unified quantitation. For 30 pesticides, such as β-endosulfan, dimethoate, and oxyfluorfen, only individual matrices fell within this interval, exhibiting matrix specificity. Therefore, attention should be paid to distinguishing these cases during quantitation.

160% – 170% (strong enhancement): This interval included 63 pesticides, accounting for 10.8% of the total. Cowpea, pepper, and carrot were the most prominent matrices, and paclobutrazol and dicloran exhibited the most significant effects. The strong enhancement in this interval is mainly attributed to the synergistic interaction between the high pigment and high wax content of the matrices and the polar structures of the pesticides.

Method optimization strategy: For initial screening using solvent-prepared calibration standards, the quantitative bias can be controlled within ±15% by introducing matrix effect correction factors (MF) for conversion according to the intervals (0.80 for 120% – 130%, 0.75 for 130% – 140%, 0.70 for 140% – 150%, 0.65 for 150% – 160%, and 0.60 for 160% – 170%). Meanwhile, selecting simple and universal matrices according to the intervals can balance detection efficiency and accuracy. These matrices are bean sprout, cucumber, mango, carrot, and cowpea, corresponding to the five different enhancement intervals, respectively. This tiered management strategy can provide technical support for the standardization of quality control and the unification of regulatory practices in multi-pesticide residue detection.

Confirmation of results using real samples

The established method was applied to the full-scope screening of the above 88 pesticides in 20 commercially available vegetable samples and 10 fruit samples that had tested positive by rapid screening methods. The following positive results were obtained: chlorpyrifos was detected in one Chinese chive sample at 0.053 mg/kg; fenthion was detected in three green pepper samples at 0.33, 0.62, and 0.089 mg/kg, respectively; phorate was detected in two carrot samples at 0.12 and 0.052 mg/kg; difenoconazole was detected in two sugar orange samples and two Little Tainong mango samples at 0.39, 0.92, 0.25, and 0.58 mg/kg, respectively; and bifenthrin was detected in one mandarin orange sample at 0.056 mg/kg. The levels of all other pesticide components were below their respective maximum residue limits. The application of this method for the rapid screening confirmation of samples that tested positive by rapid testing methods during market inspections serves two purposes. On the one hand, it eliminates the risk of erroneous conclusions caused by falsepositive results from test strip detection. On the other hand, it provides supervision direction for law enforcement, saves time, and improves overall efficiency.



Matrix type	Three types of matrix effects// %		
	Relatively strong matrix effect	Weak matrix effect	Medium matrix effect
Bean sprout	14.8	13.6	71.6
Ginger	59.1	4.5	36.4
Scallion	31.8	10.2	58.0
Carrot	65.9	4.5	29.5
Cucumber	22.7	9.1	68.2
Romaine lettuce	23.9	10.2	65.9
Cowpea	62.5	8.0	29.5
Shanghai bok choy	20.5	11.4	68.2
Celery	26.1	17.0	56.8
Pepper	68.2	4.5	27.3
Chinese chive	68.2	6.8	25.0
Dragon fruit	12.5	11.4	76.1
Mango	21.6	8.0	70.5
Banana	34.1	10.2	55.7
Sweet orange	13.6	18.2	68.2
Pomelo	13.6	10.2	76.1
Tangerine	14.8	17.0	68.2
Sugar mandarin	22.7	19.3	58.0
Orange	17.0	18.2	64.8

Fig. 6 Distribution of 88 pesticide matrix effects among 19 types of matrices

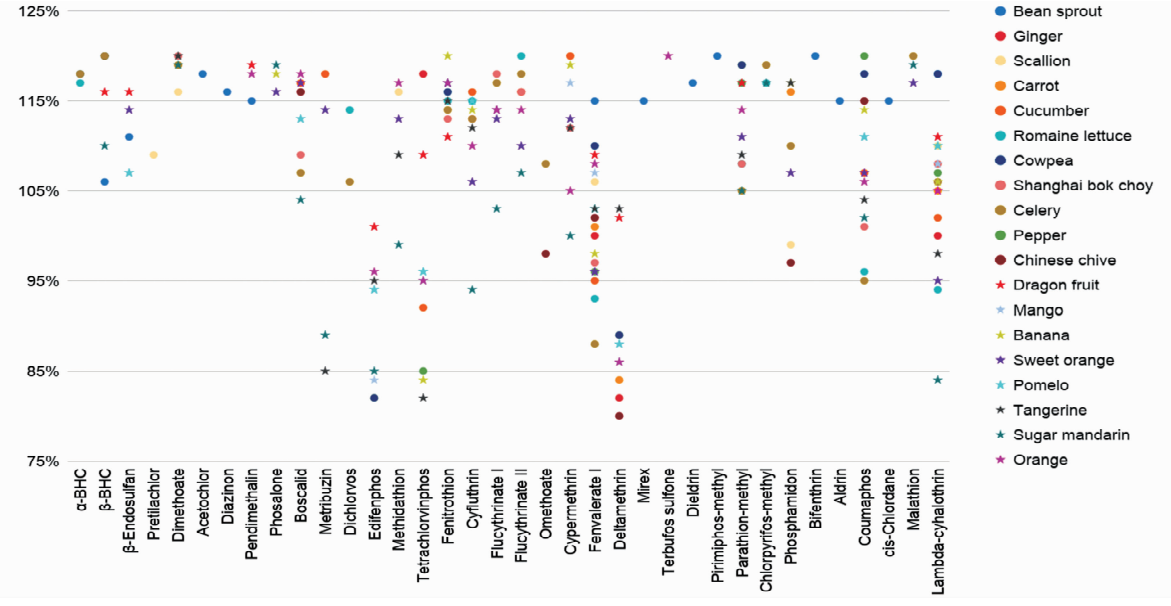


Fig. 7 Distribution of weak matrix effects of 36 pesticides in 19 matrices

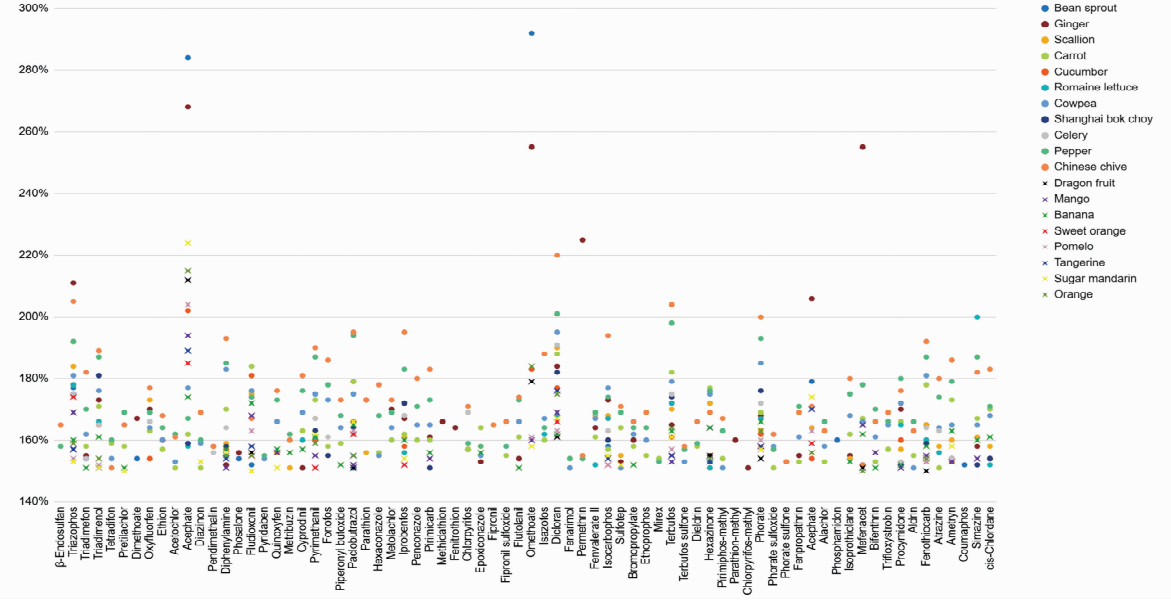


Fig. 8 Distribution of strong matrix enhancement effects of 74 pesticides in 19 matrices

Conclusion and Discussion

The matrix effect is a core interference factor that limits the accuracy of quantitative pesticide residue detection in fruits and vegetables by GC-MS, directly affecting the accuracy and reliability of the test results. Aiming at high-throughput detection of multipesticide residues in fruits and vegetables, this study systematically constructed a matrix effect evaluation matrix covering 88 pesticides and 19 fruit and vegetable matrices, and clarified the distribution characteristics of the matrix effects of different pesticides in multiple sample matrices. The experimental results showed that the tested pesticides overall exhibited predominantly matrix enhancement effects. Among them, 54.9% of the pesticides showed a medium matrix enhancement effect, 32.3% showed a relatively strong matrix enhancement effect, 8.6% showed a relatively weak matrix enhancement effect, and only 4.2% of the pesticides showed a matrix suppression effect. Among the tested pesticide components, paclobutrazol, triazophos, phorate, dicloran, terbufos, and fenothiocarb exhibited the most significant matrix enhancement effects. In contrast, six pesticides, namely, edifenphos, tetrachlorvinphos, deltamethrin, fenvalerate I, lambd-acyhalothrin, and metribuzin, exhibited obvious matrix suppression effects. From the perspective of matrix type differences, the matrix effect characteristics of bean sprout and dragon fruit were relatively simple. In both matrices, medium-intensity matrix enhancement effects predominated, the proportions of extreme strong and weak matrix effects were relatively low, and no matrix suppression effects occurred. Therefore, these two matrices are suitable for use as universal detection matrices for crosscategory multi-pesticide residue analysis in fruits and vegetables and can be recommended as preferred representative matrices. Pepper, Chinese chive, carrot, and cowpea exhibited prominent relatively strong matrix enhancement effect characteristics and can serve as typical representative matrices for the strong matrix effect interval. Based on the distribution patterns of matrix effects of different intensities, this study further screened universal matrices suitable for the five levels of matrix enhancement intervals, with bean sprouts, cucumber, mango, carrot, and cowpea corresponding to the relatively weak, weak, medium, strong, and very strong matrix effect levels, respectively.

Combining the practical detection requirements for high-throughput screening of multi-pesticide residues, this study established a differentiated quantitative detection strategy. Depending on the testing scenario, either the universal matrix calibration method or the solvent calibration standard initial screening method can be flexibly selected. For the solvent calibration standard initial screening mode, a tiered correction system with interval-specific matrix effect correction factors (MF) was further constructed. Specifically, correction factors of 0.80, 0.75, 0.70, 0.65, and 0.60 were assigned to the matrix effect intervals of 120% – 130%, 130% – 140%, 140% – 150%, 150% – 160%, and 160% – 170%, respectively. This approach can effectively offset systematic deviations during the detection process and improve quantitative accuracy.

This study systematically elucidated the distribution patterns of matrix effects in a multi-matrix, multi-pesticide system and improved the matrix adaptation system for GCMS detection of multi-pesticide residues in fruits and vegetables, providing a solid theoretical basis for establishing an efficient and universal quantitative detection model for multi-matrix pesticide residues. Meanwhile, the tiered correction method and matrix selection criteria established in this study provide a practical technical solution for the standardization of quality control and the unification of regulatory practices in pesticide residue detection in fruits and vegetables. This study has significant practical value for improving the standardization and accuracy of quality and safety testing of plant-derived foods.

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This is distinct from conventional chemotherapeutic agents. DC can exert indirect effects by modulating immune cell functions, a feature that gives it a potential advantage in the field of cancer immunotherapy. Moreover, previous studies have reported that DC has protective effects against liver fibrosis and acute liver injury[10,17-19], suggesting that DC possesses both hepatoprotective and anti-tumor properties, making it particularly suitable for the treatment of liver cancer in the context of cirrhosis. In addition, DC is derived from natural sources with high safety. In this study, cell viability assays showed no significant cytotoxicity toward THP-1 cells, laying a foundation for its further application.

In summary, DC can inhibit the proliferation, migration, invasion, and EMT ability of liver cancer cells by suppressing M2-like polarization of macrophages, demonstrating its potential as a novel anti-liver cancer drug candidate. Further studies will validate the anti-liver cancer activity of DC in animal models and utilize transcriptomic or proteomic approaches to elucidate its key signaling pathways, thereby providing more robust experimental evidence for the clinical transformation of DC.

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