The Extraction of Pesticides from Color Additives

Abstract

Color additives are used in food manufacturing, as certain colors are often associated with certain flavors. Through their history, color additives have often been contaminated with other compounds to decrease their cost, making their monitoring critical. In the present day, pesticide content is a concern with color additives because many are of natural origin. To measure pesticide content, typically foods are extracted using QuEChERS sample preparation, which are manual and often wasteful and time-consuming extractions. In this work, the EDGE® , an automated extraction system by CEM, was used to extract pesticides from various color additives provided by the AOAC Color Additives Community. The extraction efficiency was assessed for the various matrices, and it was found that the EDGE extracted pesticides from color additives with good recoveries and standard deviations. The EDGE is a good choice for laboratories seeking to automate pesticide extractions.

Introduction

Color additives to food are an integral part of the food industry, as consumers associate certain colors with particular foods and flavors. In the past, food color additives have often been adulterated by other substances to make them cheaper. Because of this, there is an extensive history of monitoring their safety to prevent additives from causing harm. A modern concern with food color additives is the level of pesticides they contain. Many food color additives originate as natural products that were grown commercially, making them susceptible to pesticide contamination. Furthermore, many color additives are extracts that were concentrated, potentially increasing the concentration of pesticides. In order to analyze these additives for pesticides, typically, extraction techniques like QuEChERS are used to extract the sample. The QuEChERS sample preparation approach traditionally uses acetonitrile and salts to extract pesticides from food matrices. It is manual and generates several pieces of waste per sample. Thus, alternative means of extraction that generate less waste and are automated are needed.

In this application note, the EDGE, an automated extraction system by CEM, was utilized to extract pesticides provided by Restek from several food color additives with different consistencies (powders and oleoresins), including bixin, curcumin, sodium copper chlorophyllin, norbixin, cheese color, carrot oleoresin, annatto, and paprika extract. Often, these matrices are regarded as difficult. Using standard addition spikes, the EDGE was shown to extract both dry and oleoresin color additives with high extraction recoveries, 60-125%, and favorable standard deviations of less than 20%.

These extraction recoveries and standard deviations meet the requirements proposed by the AOAC Color Additives community, making the EDGE a potential solution for automated pesticide extractions.

Materials and Methods

Color additive samples (bixin, curcumin, sodium copper chlorophyllin, norbixin, cheese color, carrot oleoresin, annatto, and paprika extract) were provided by the AOAC Color Additives Community. The pesticide spike mix was provided by Restek. Acetonitrile, acetic acid, acetone, methanol, and petroleum ether were purchased from Sigma. Other supplies were provided by CEM.

Each sample type was prepared slightly differently using the sample mass, spike mass, and additional sorbents indicated in **Table 1** (page 3). The samples, along with their additional sorbents as indicated, were weighed directly into the Q-Cup prepared with the S1 Q-Disc Stack. Each sample was spiked with the indicated amount of each pesticide from a custom mix provided by Restek through the AOAC Color Additives Community. Samples were prepared in triplicate. Each extraction vial was also prepared with a Q-Shield. The prepared Q-Cups and extraction vials were placed in an EDGE rack, and the rack was placed into the EDGE. EDGE methods were ran with the matrices indicated in **Table 2** (page 3) using the extraction solvent acetonitrile with 1.0% acetic acid (v/v) and the agitation directed in **Table 1** (page 3).

Analysis

A portion of 1 mL from each extract was transferred to an amber vial, and 10 µl of the sample were injected without dilution onto a Waters Acquity H Class UPLC system attached to a Waters XEVO TQD mass spectrometer. The pesticides were separated using a Restek Raptor ARC-18 column (2.7 µm, 100 x 2.1 mm) with the mobile phases 4 mM ammonium formate with 0.1% formic acid in water (mobile phase A) and 4 mM ammonium formate with 0.1% formic acid in methanol (mobile phase B) at the flow rate of 0.25 mL/min using the program indicated in **Table 3** (page 3). They were also monitored using their respective MRM transitions.

Results

Each spiked sample was compared to a post-extraction spiked sample to assess recovery data. The recovery data for each powdered matrix and norbixin are presented in **Table 4** (page 4). For these matrices, 54 compounds were screened, and the acceptable recovery range was 60%-125%.

For bixin, of these compounds, 53 compounds had recovery values within the acceptable range, and only one compound had a standard deviation greater than 20%. For curcumin, 49 compounds had recovery values within the acceptable range, and all compounds had favorable standard deviations. For sodium copper chlorophyllin, 53 compounds had recovery values within the acceptable range, and only one compound had a standard deviation greater than 20%. For norbixin, all 54 compounds had recovery values within the acceptable range, and only one compound had a standard deviation greater than 20%. Thus, the EDGE was able to extract pesticides from powdered color additives.

The recovery data for each oleoresin are presented in **Table 5** (page 5). For each oleoresin, 58 pesticides were analyzed. For cheese color, 54 compounds had recovery values within the acceptable range, and 51 compounds had standard deviation values below 20%. For carrot oleoresin, 56 pesticides had recovery values within the acceptable range, and 53 compounds had standard deviation values below 20%. For annatto, 55 compounds had recovery values within the acceptable range, and 53 compounds had standard deviation values below 20%. For paprika extraction, the recovery values for 57 pesticides were within the acceptable range, and 53 compounds had standard deviation values below 20%. Based on these data, it was shown that the EDGE can extract oleoresins, which are regarded as difficult to work with, with high recoveries and favorable standard deviations.

Conclusion

Color additives are an important tool for the food industry. Because they often have agricultural origins, pesticide testing is needed. Typically, QuEChERS sample preparation is used to extract pesticides from food, but it is a very manual process. In this work, the EDGE was used to extract pesticides from various color additives with different consistencies, and the recoveries and standard deviations for each matrix were assessed. It was found that the EDGE obtained recoveries and standard deviations for the pesticides assessed in the acceptable range proposed by the AOAC Color Additives Community. Thus, the EDGE is an ideal choice for laboratories looking to extract pesticides from color additives in an automated fashion.

Table 1. Q-Cup Preparation and Agitation in the EDGE

Matrix	Sample Mass	Spike Mass	Additional Sorbents	Agitation
Bixin	1 g	2500 ng	No	No
Curcumin	1 g	2500 ng	No	No
Sodium Copper Chlorophyllin	1 g	2500 ng	No	No
Norbixin	1 g	2500 ng	1 g Q-Matrix Hydra below sample	No
Cheese Color	1 g	5000 ng	1 g Q-Matrix Hydra below sample	Yes, 1 minute
Annatto	0.75 g	5000 ng	0.75 g DE* below sample	Yes, 1 minute
Paprika Extract	0.5 g	5000 ng	0.5 g DE* below sample	Yes, 1 minute
Carrot Oleoresin	1 g	5000 ng	1 g DE* below sample	Yes, 1 minute

*DE: Diatomaceous Earth

Table 3. UPLC Gradient Used to Separate Pesticides

Time (minutes)	% A	% B
Initial	95	5
2	40	60
4	25	75
14.5	0	100
15.5	0	100
15.51	95	5
18.01	95	5

Table 2. EDGE Method Parameters

	Matrix	Bixin	Curcumin	Sodium Copper Chlorophyllin	Norbixin	Cheese Color	Annatto	Paprika Extract
Cycle 1	Top Add (mL)	15	15	15	15	15	15	15
	Bottom Add (mL)	0	0	0	0	0	0	0
	Rinse (mL)	10	10	10	10	10	10	10
	Temperature (°C)	40	40	40	40	40	40	40
	Hold Time (mm:ss)	3:00	3:00	3:00	3:00	3:00	4:00	4:00
Wash 1	Solvent	Methanol	Methanol	Methanol	Acetone	Acetone	Acetone	Acetone
	Volume (mL)	40	40	40	40	40	40	40
	Temperature (°C)	120	120	120	120	120	120	120
	Hold Time (mm:ss)	00:30	00:30	00:30	00:30	00:30	00:30	00:30
Wash 2	Solvent	Methanol	Methanol	Methanol	Acetone	Acetone	Petroleum Ether	Petroleum Ether
	Volume (mL)	40	40	40	40	40	40	40
	Temperature (°C)	100	100	100	100	100	80	80
	Hold Time (mm:ss)	00:30	00:30	00:30	00:30	00:30	00:30	00:30
Wash 3	Solvent	Extraction Solvent	Extraction Solvent	Extraction Solvent	Extraction Solvent	Extraction Solvent	Extraction Solvent	Extraction Solvent
	Volume (mL)	40	40	40	40	40	40	40
	Temperature (°C)	40	40	40	40	40	40	40
	Hold Time (mm:ss)	- -:- -	- -:- -	- -:- -	- -:- -	- -:- -	- -:- -	- -:- -

Table 4. Recovery Data for Bixin, Curcumin, Sodium Copper Chlorophyllin, and Norbixin

	Bixin		Curcumin		Sodium Copper Chlorophyllin		Norbixin	
Compound	**% Recovery**	**% RSD**	**% Recovery**	**% RSD**	**% Recovery**	**% RSD**	**% Recovery**	**% RSD**
Methamidophos	86.9%	2.0%	102.3%	9.7%	55.5%	4.8%	97.9%	10.1%
Methomyl	97.0%	2.2%	88.5%	3.8%	81.8%	10.8%	83.6%	5.2%
Acephate	95.9%	4.3%	91.9%	4.1%	66.1%	1.6%	95.2%	14.7%
Propamocarb	89.2%	7.6%	78.0%	10.8%	42.7%	4.8%	72.1%	8.8%
Carbendazim	128.5%	7.0%	87.5%	4.3%	85.3%	8.2%	80.0%	17.4%
Pyrimethanil	91.0%	4.0%	95.5%	10.6%	72.4%	7.3%	87.9%	7.6%
Carbaryl	102.3%	3.3%	93.5%	8.0%	100.7%	12.4%	86.7%	10.8%
Dinotefuran	92.0%	2.4%	81.5%	9.4%	77.4%	5.5%	80.4%	8.9%
Omethoate	72.7%	5.5%	82.0%	15.0%	70.7%	12.9%	101.7%	12.2%
Acetamiprid	90.1%	1.1%	98.5%	4.3%	83.0%	4.3%	80.4%	7.2%
Cyprodinil	79.2%	8.2%	90.1%	6.1%	82.5%	13.3%	87.7%	14.0%
Dimethoate	92.7%	4.9%	90.9%	9.0%	96.8%	8.3%	80.3%	12.0%
Flonicamid	70.0%	19.7%	151.5%	41.0%	98.8%	14.6%	94.7%	19.4%
Fludioxonil	105.0%	2.2%	102.5%	12.9%	78.5%	4.7%	103.6%	11.6%
Linuron	111.0%	11.5%	81.4%	1.3%	99.8%	5.7%	96.2%	7.1%
Thiacloprid	125.1%	6.3%	81.3%	7.7%	96.9%	11.5%	110.6%	7.2%
Imidacloprid	84.2%	2.4%	98.3%	5.0%	84.8%	10.8%	84.1%	16.7%
Sulfoxaflor	85.7%	5.0%	81.5%	6.0%	92.2%	5.8%	73.7%	7.1%
Metalaxyl	102.7%	4.7%	103.7%	13.0%	89.9%	17.0%	97.8%	15.1%
Myclobutanil	101.8%	4.8%	91.9%	8.5%	77.4%	8.9%	101.3%	11.1%
Flupyradifurone	115.0%	0.7%	91.8%	9.4%	77.9%	16.2%	91.0%	12.1%
Flutriafol	123.2%	25.3%	117.4%	10.1%	94.7%	8.3%	84.4%	14.8%
Fenhexamid	100.4%	14.8%	144.8%	12.2%	62.0%	11.0%	100.6%	16.2%
Diazinon	98.8%	3.7%	99.3%	1.5%	89.4%	2.2%	98.1%	6.9%
Buprofezin	97.1%	6.8%	114.3%	4.8%	93.5%	2.7%	100.1%	2.7%
Quinoxyfen	99.0%	4.3%	100.9%	2.5%	70.4%	10.9%	112.7%	14.3%
Tebuconazole	92.4%	2.8%	85.0%	3.2%	76.8%	4.6%	106.5%	8.4%
Diflubenzuron	95.5%	10.2%	93.0%	1.9%	85.4%	3.9%	100.4%	4.8%
Pyriproxyfen	99.3%	11.7%	96.9%	4.9%	87.0%	1.0%	93.9%	8.5%
Cyazofamid	110.6%	4.5%	90.8%	5.9%	36.7%	0.5%	101.6%	5.1%
Malathion	86.1%	6.9%	77.2%	5.9%	86.8%	5.5%	96.4%	25.6%
Fenbuconazole	92.8%	3.9%	93.6%	5.8%	88.6%	5.9%	95.4%	9.0%
Propiconazole	94.8%	5.9%	85.5%	12.6%	86.0%	4.1%	99.4%	7.3%
Boscalid	89.4%	7.5%	108.1%	2.6%	95.3%	13.6%	117.8%	8.2%
Triflumizole	76.1%	2.3%	78.3%	4.2%	77.3%	0.7%	102.9%	6.9%
Piperonyl Butoxide	97.6%	9.8%	98.2%	3.6%	88.8%	3.0%	100.2%	7.1%
Etoxazole	103.3%	7.0%	100.8%	4.2%	89.0%	1.8%	86.0%	16.9%
Pyridaben	68.1%	19.1%	76.3%	12.7%	89.4%	21.7%	104.5%	7.4%
Methoxyfenozide	90.7%	5.0%	100.3%	5.1%	98.0%	5.0%	97.1%	2.5%
Spirotetramat	101.8%	4.0%	94.5%	3.6%	83.8%	7.8%	105.9%	9.4%
Fluxapyroxad	97.3%	5.5%	91.5%	6.3%	98.0%	4.2%	104.0%	5.5%
Pyraclostrobin	99.0%	6.1%	89.5%	6.5%	92.3%	1.6%	102.4%	5.5%

Dimethomorph isomers	148.1%	8.1%	99.9%	2.3%	100.8%	3.1%	109.4%	13.0%
Fluopyram	115.9%	6.2%	98.1%	19.7%	98.1%	11.1%	104.4%	7.8%
Azoxystorbin	89.2%	4.9%	93.1%	9.5%	87.0%	3.7%	105.9%	8.8%
Difenconazole isomer	90.4%	7.2%	92.0%	5.6%	93.5%	1.9%	97.4%	7.1%
Trifloxystrobin	104.9%	19.7%	154.0%	12.0%	95.2%	6.7%	103.2%	4.1%
Spirodiclofen	96.8%	5.2%	93.1%	3.9%	93.4%	3.6%	102.3%	6.2%
Mandipropamid	95.9%	1.7%	101.9%	11.6%	88.3%	1.3%	94.9%	6.3%
Fipronil	98.4%	12.8%	153.1%	3.0%	125.1%	3.6%	108.2%	7.7%
Chlorantraniliprole	103.6%	12.1%	86.3%	9.9%	101.8%	9.3%	99.7%	18.1%
Novaluron	106.0%	10.2%	90.6%	7.8%	88.9%	9.9%	119.4%	3.0%
Indoxacarb	92.4%	16.0%	157.3%	17.4%	101.5%	3.8%	107.5%	13.0%
Spinetoram	101.9%	2.9%	101.2%	1.0%	104.3%	0.5%	90.2%	16.4%

Table 5. Recovery Data for Cheese Color, Carrot Oleoresin, Annatto, and Paprika Extract

	Cheese Color		Carrot Oleoresin		Annatto		Paprika Extract	
Compound	**% Recovery**	**% RSD**	**% Recovery**	**% RSD**	**% Recovery**	**% RSD**	**% Recovery**	**% RSD**
Methamidophos	89.0%	15.9%	88.89%	10.96%	98.0%	5.2%	78.2%	14.3%
Methomyl	86.7%	9.0%	90.92%	17.60%	77.9%	15.3%	84.1%	19.2%
Acephate	86.9%	13.0%	88.29%	16.93%	72.5%	16.7%	77.5%	15.4%
Propamocarb	75.1%	11.0%	68.50%	12.97%	14.8%	2.8%	53.8%	7.6%
Carbendazim	79.1%	6.0%	87.18%	16.64%	75.2%	13.8%	81.1%	20.2%
Pyrimethanil	138.3%	40.3%	82.20%	13.33%	85.0%	16.1%	86.9%	14.5%
Thiabendazole	81.7%	12.4%	75.33%	10.72%	60.7%	10.6%	69.2%	15.5%
Carbaryl	89.5%	19.2%	96.67%	31.01%	79.0%	22.5%	88.8%	18.7%
Dinotefuran	78.7%	13.1%	80.03%	12.39%	71.4%	15.4%	79.5%	13.9%
Omethoate	101.7%	17.5%	95.16%	5.16%	102.5%	9.6%	91.4%	10.2%
Acetamiprid	93.6%	7.7%	85.18%	11.64%	79.0%	13.0%	84.7%	16.1%
Cyprodinil	102.8%	14.6%	123.61%	26.59%	98.4%	16.1%	70.8%	14.2%
Flonicamid	97.3%	7.9%	83.44%	11.38%	66.9%	15.6%	78.1%	16.3%
Dimethoate	95.9%	14.7%	91.92%	11.86%	92.5%	18.0%	77.6%	16.6%
Linuron	104.5%	32.9%	116.29%	27.95%	99.6%	19.4%	84.4%	27.6%
Thiacloprid	91.7%	10.1%	87.66%	10.37%	78.5%	14.4%	83.1%	17.2%
Imidacloprid	93.4%	7.3%	97.60%	8.50%	74.2%	5.1%	77.5%	18.8%
Sulfoxaflor	92.8%	10.6%	85.83%	14.60%	82.9%	20.1%	83.9%	18.4%
Metalayxl	116.3%	12.9%	93.99%	14.02%	79.3%	13.7%	94.8%	16.5%
Flupyradifurone	88.1%	11.6%	89.91%	13.00%	72.7%	13.2%	77.4%	16.4%
Myclobutanil	104.4%	15.4%	124.90%	21.21%	104.0%	17.1%	65.8%	19.4%
Thiamethoxan	81.6%	11.0%	88.63%	7.79%	81.5%	10.9%	84.8%	17.4%
Imazalil	89.9%	7.0%	76.66%	7.99%	61.2%	5.5%	77.8%	18.3%
Flutriafol	83.3%	12.4%	77.36%	6.22%	65.2%	6.7%	74.9%	17.3%
Bifenazate	86.9%	19.4%	100.51%	26.78%	56.9%	8.6%	70.2%	3.3%
Fenhexamid	77.1%	10.1%	129.13%	11.46%	74.0%	20.0%	66.4%	13.9%
Diazinon	98.3%	7.9%	85.93%	13.13%	89.6%	23.0%	97.6%	16.9%
Buprofezin	98.0%	5.8%	89.07%	11.04%	80.8%	13.8%	78.1%	20.2%
Quinoxyfen	100.5%	8.7%	78.81%	13.57%	79.6%	12.8%	77.6%	18.4%

Tebuconazole	104.3%	13.8%	62.58%	15.52%	84.4%	28.3%	105.8%	2.6%
Diflubenzuron	119.7%	19.4%	69.07%	3.79%	82.3%	14.6%	89.0%	26.1%
Pyriproxyfen	103.2%	6.4%	85.00%	10.89%	80.1%	13.7%	94.1%	17.7%
Cyazofamid	59.9%	10.9%	43.05%	6.95%	110.3%	31.7%	84.8%	24.8%
Malathion	71.0%	15.0%	100.95%	19.02%	80.0%	14.9%	66.3%	17.4%
Fenbuconazole	101.9%	61.1%	95.65%	18.66%	101.4%	13.5%	68.7%	17.1%
Propiconazole isomers	91.0%	7.2%	92.81%	6.86%	80.5%	13.0%	84.1%	20.4%
Boscalid	94.7%	11.9%	88.03%	11.01%	114.4%	16.1%	70.9%	17.1%
Triflumizole	90.8%	7.6%	100.14%	17.69%	76.4%	11.7%	74.6%	15.5%
Piperonylbutoxide	100.4%	8.0%	102.12%	20.83%	78.0%	12.0%	68.6%	15.6%
Etoxazole	91.0%	6.9%	102.28%	20.72%	79.6%	12.6%	75.8%	16.5%
Pyridaben	63.1%	3.5%	83.78%	12.24%	75.3%	13.0%	87.8%	20.3%
Methoxyfenozide	79.0%	17.3%	135.40%	16.90%	92.2%	60.9%	90.0%	11.0%
Spirotetramat	76.4%	19.2%	68.47%	9.43%	96.1%	12.5%	69.4%	21.7%
Fluxapyroxad	79.3%	21.2%	71.18%	6.86%	83.5%	12.5%	63.6%	11.9%
Dimethomorph isomers	86.4%	19.0%	90.98%	9.85%	61.9%	13.1%	105.2%	13.6%
Pyraclostrobin	97.3%	6.6%	91.72%	17.89%	80.5%	12.7%	82.3%	18.3%
Fluopyram	134.5%	64.3%	125.64%	6.26%	97.6%	17.5%	64.5%	13.1%
Azoxystrobin	96.9%	4.1%	79.67%	14.64%	88.2%	13.6%	124.1%	20.5%
Difenconazole isomers	78.6%	6.7%	86.36%	19.46%	76.0%	14.5%	74.9%	18.6%
Trifloxystrobin	99.1%	7.7%	87.51%	18.69%	79.5%	13.3%	77.5%	19.4%
Spirodiclofen	77.0%	6.4%	86.00%	18.44%	77.1%	14.1%	93.6%	18.9%
Mandipropamid	60.8%	3.8%	102.05%	17.57%	113.5%	9.9%	64.2%	14.4%
Fipronil	73.2%	20.2%	86.99%	19.86%	80.5%	16.1%	77.4%	20.6%
Chlorantraniliprole	97.0%	23.5%	91.09%	15.43%	78.8%	15.0%	93.2%	18.9%
Novaluron	59.7%	5.3%	95.91%	15.85%	78.6%	17.2%	100.4%	15.6%
Indoxacarb	64.5%	4.4%	87.07%	12.56%	79.0%	12.6%	94.5%	18.8%
Flubendiamide	105.6%	59.9%	81.35%	19.30%	74.7%	6.2%	87.3%	17.4%
Spinetoram	85.9%	7.6%	77.24%	9.68%	50.5%	4.7%	79.6%	16.9%

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