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Food fraud in oregano: Pesticide residues as adulteration markers

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ABSTRACT

Oregano, a widely used and popular herb, is particularly vulnerable to fraud. Less valued plants, adulterants that are often used for dilution, may introduce into this commodity additional contaminants such as pesticide residues. In this study, more than 400 pesticides were screened in a representative set of 42 genuine and 34 adulterated dried oregano samples collected from various locations across Europe. The results obtained by advanced mass spectrometry-based methods, showed, that some pesticide residues could be detected in virtually all tested samples, nevertheless, on average, higher contamination was found in the adulterated oregano samples. Increased incidence of insecticides such as cyfluthrin, permethrin and cyhalothrin was typical for these samples, moreover, pyriproxyfen was detected exclusively in adulterated samples. Thus, based on a critical assessment of pesticide profiles, suspected adulterated oregano samples can be selected for follow up authenticity testing.

1. Introduction

Herbs and spices are high value food commodities, playing an important role as ingredients in a multitude of foods, beverages, and other products associated with health and beauty. Over the last few decades, the global herb and spice market has grown considerably, and was worth $12 billion in 2012, and is expected to increase at an annual growth rate of 4.8% to reach an estimated value of US $16.6 billion by 2019. The European Union (EU) market is the second largest trade for herbs and spices, amounting to 520,000 tonnes in 2013 with a value of € 1.8 billion (The 3rd CARIFORUM-EU Business Forum, 2015). Motivated by the high demand and prices, as well as the complexity of the supply chains, the potential presence of accidental adulterants or the intentional addition of fraudulent components (e.g. artificial additives, leaves from other plants or foreign species), must be taken into consideration in these commodities (Reinholds, Bartkevics, Silvis, van Ruth, & Esslinger, 2015).

Oregano is a herb very frequently used for culinary purposes. The *Origanum* genus involves different botanical genera from both Mediterranean and Mexican origin. Only some specific genera (*Origanum vulgare L. ssp. hirtum* and *Origanum onites L.*) are considered at the European market as true oregano with some restrictions on the level of impurities (extraneous materials max. 2%) (ESA, 2015) whereas other markets allow leaves of all *Origanum* genus with other limitations specified in ISO/FDIS 7925 (ISO 7925, 2015) and the American Spice Trade Association guidelines (ASTA, 2015).

Oregano adulteration has been investigated in a number of studies, in which various methods were used for the detection of adulterated samples (Galvin-King at al., 2017). For instance for identification of adulterants in dried commercial oregano a method based on sequence-characterized amplified region makers (SCARs), was employed by Marieschi, Torelli, Poli, Sacchetti, and Bruni (2009), Marieschi, Torelli, Poli, Bianchi, and Bruni (2010), Marieschi, Torelli, Bianchi, and Bruni (2011a,b). Alternatively, methods such as gas or liquid chromatography coupled to mass spectrometry (GC-MS or LC-MS/MS), were developed by Wielogorska et al. (2018), Bononi, Fiordaliso, and Tateo (2010), and Bononi and Tateo (2011) to identify the presence of olive leaves used as a adulterants agent in ground oregano. More recently, a comprehensive strategy based on the application of both, Fourier-Transform Infrared spectroscopy (FTIR) and liquid chromatography high resolution mass spectrometry (LC-HRMS) as a two-tier approach to screen oregano adulteration was reported. In this way, 24% of the samples tested were found to identify as adulterated. The scale of adulteration (based on the sample weight) ranged from 30% to over 70%, and two samples contained no oregano present at all. According to these studies, leaves originated from olive trees, myrtle, sumac, cistus or hazelnut leaves,

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among others, can be found in fraudulent oregano samples. The presence of these impurities from various plant sources, not only affects the quality of the final product but also may introduce pesticide residues, moreover, some of them might not be registered for oregano treatment, thereby further compromising the chemical safety of such products.

The EU Regulation No. 396/2005 (Regulation (EC) No 396/2005, 2005) and amendments lay down stringent residue levels of pesticide concentrations in herbs like oregano, which in most cases are equal to 0.01 mg/kg where no specific MRLs are set. However, there is insufficient data regarding pesticide residues in herbs, particularly in oregano, since the matrix complexity of such products, containing mixtures of essential oils, phytosterols, pigments and many other plant-derived components interfering with chromatographic analysis still poses a great challenge for many laboratories (Nantia, Moreno-González, Manfo, Gámiz-Gracia, & García-Campaña, 2017).

To overcome these drawbacks, sample preparation strategies based on liquid-solid extraction (LSE) (Łozowicka et al., 2014; Rao, Kumarmeena, & Ruknuddin, 2011; Hajjo, Afifi, & Battah, 2007; Wang, Jin, Ma, Lu, & Lin, 2011), microwave-assisted extraction (MAE) (Wan, Mao, Yan, Shen, & Wu, 2010), matrix solid phase dispersion (MSPD) (Łozowicka et al., 2014), solid phase extraction (SPE) (Tuzimski, 2010) or dispersive SPE (dSPE) (Zhang, 2010) have been used for determination of pesticide residues in herbal products. In the recent decade, QuEChERS has become the most frequently applied extraction technique for this purpose, in combination with GC-MS (MS/MS) or LC-MS/MS (Nantia et al., 2017; Dai, Ren, He, & Huo, 2011; Esturk, Yakar, & Ayhan, 2014; Chen, Cao, & Liu, 2011; Sadowska-Rociek, Surma, & Cieślik, 2013; Nguyen, Lee, Lee, & Lee, 2010; Rajski et al., 2013). Although many of these studies are focused on a selected number or groups of pesticides, some reported methods propose the multiresidue analysis of pesticides (mostly GC amenable compounds) in tea or medical herbs (Wang et al., 2011; Rajski et al., 2013; Cajka et al., 2012). However, in spite of the efforts made so far, no analytical method has been reported to simultaneously screen a large number of pesticides in oregano samples. Only recently LC-Orbitrap based method concerned with pesticide residues in spices has been published by Goon et al. nevertheless, oregano is not on the list (Goon et al., 2018).

In this study, we investigated the potential difference of pesticide residues pattern in genuine oregano samples and those adulterated by the addition of other plant materials. Mass spectrometry-based methods enabling to determine more than 400 pesticide residues were used for this purpose. It was assumed that plant-based adulterant added to oregano may introduce additional/different contamination into the herb samples. Based on the knowledge of the pesticide profile in particular sample, it could be possible to identify suspected samples thus protect consumers not again fraud but also reduce health risk.

2. Experimental

2.1. Chemicals and materials

Pesticide standards were supplied by Dr. Ehrenstorfer GmbH (Augsburg, Germany) and Sigma–Aldrich (Taufkirchen, Germany). Acetonitrile, hexane, and methanol were obtained as high purity solvents for pesticide residue analysis from Merck (Darmstadt, Germany) and Supelco (Bellefonte, PA, USA). Acetic acid (HPLC grade), ammonium formate (≥99%), and formic acid and triphenyl phosphate (TPP) were from Sigma–Aldrich (Taufkirchen, Germany). Anhydrous magnesium sulphate ($MgSO_4$) was purchased from Fluka (Buchs, Germany), and sodium chloride (NaCl) from Penta (Chrudim, Czech Republic). Sorbent primary–secondary amine (PSA, Bondesil (40 μm)) was obtained from Agilent Technologies (USA). Deionized water was purified using a Milli-Q system (Millipore; Bedford, MA, USA).

A pesticides standard solution (2 μg mL^{-1}) and triphenyl phosphate (TPP, 5 μg mL^{-1}) standard solution were prepared in acetonitrile. Stock and working solutions were stored in a freezer at −20 °C protected from light.

2.2. Oregano samples

Seventy-six samples of dry oregano with the known origin, manufacturer and traceability, sourced from sixteen different countries all over the world were obtained during the years 2015 and 2016. Other samples batches were also acquired from online retailers from both EU and non EU countries. Oregano samples were kept in their original packaging at room temperature until their analysis. The samples were milled to a homogeneous powder on a PM-100 Retsch Planetary Ball Mill (Haan, Germany).

2.3. QuEChERS procedure

A modified version of the sample preparation procedure described by Cajka et al. (2012), originally developed for GC amenable residues, was applied for the extraction of pesticides from oregano. A portion of 1 g of homogenized oregano sample was weighed into a 50 mL polypropylene centrifuge tube. When it was necessary, the sample was fortified with the appropriate volume of a mix solution of the target analytes (2 μg mL^{-1}) to achieve the desired final concentration. The spiked sample was allowed to stand for 30 min at room temperature. Subsequently, 10 mL of 1% formic acid in water (*v/v*) was added, and the sample was left to soak for matrix hydratation. After 30 min, 10 mL of acetonitrile were added, and the mixture was shaken vigorously for 1 min by hand. A portion of 4 g of anhydrous $MgSO_4$ and 1 g of NaCl was added and the tube was immediately shaken for 1 min by hand. After the addition of 100 μL of the triphenyl phosphate standard solution (5 μg mL^{-1}) as the internal standard, the tube was shaken and centrifuged for 5 min at 11,180 g to induce separation of the aqueous phase from the organic phase. Before LC analysis, 5 mL of the acetonitrile extract was transferred to a 15 mL centrifuge tube and stored in the freezer for at least 2 h. An amount of 3 mL was transferred into the new tube containing 150 mg of PSA sorbent and 150 mg of anhydrous $MgSO_4$. The tube was shaken (1 min) and centrifuged for 5 min at 11,180 g. Subsequently, an aliquot of 1 mL was transferred into a vial for LC-MS/MS determination. For GC analysis, 1.5 mL of the extract was transferred to a 15 mL plastic centrifuge tube containing 1.5 mL hexane and 7.5 mL 20% NaCl (*w/w*) solution. The tube was vigorously shaken for 1 min and then centrifuged at 4020 g for 2 min. An aliquot of the upper hexane layer was transferred into a vial for GC-MS/MS analysis.

2.4. Gas chromatography-triple quadrupole mass spectrometry analysis

The GC–MS/MS analysis of 183 pesticide residues were performed using an Agilent 7890A GC system equipped with a triple quadrupole mass spectrometer 7000B (Agilent Technologies) operated in an EI mode (−70 eV). For the separation of target analytes, a capillary column HP-5MS UI column (15 m × 0.25 mm ID, 0.25 μm; Agilent Technologies) coupled with an DB-5MS UI column (0.50 m × 0.15 mm I.D., 0.15 μm; Agilent Technologies) were applied. The GC conditions used for analysis were: initial oven temperature 70 °C, held for 2.5 min, increased at 50 °C min^{-1} to 150 °C, increased at 6 °C min^{-1} to 200 °C and 16 °C min^{-1} to 280 °C, held for 4.07 min. The carrier gas was helium in constant pressure mode. Initial flow was 2.1 mL min^{-1}. A volume of 2 μL was injected using PTV injection in a solvent vent mode (vent time: 2.5 min; vent pressure: 5 psi (34.47 kPa); vent flow: 30 mL min^{-1}) with initial temperature 40 °C (2.5 min); inlet heating velocity 600 °C min^{-1} and final inlet temperature 320 °C. The multimode inlet (MMI) was cooled using carbon dioxide (CO_2). The instrument was operated in multiple reaction monitoring (MRM) mode. The MS detector set up was as follows: transfer line temperature, 280 °C; ion source temperature, 280 °C; 1st and 2nd quadrupole temperature, 150 °C. As the collision cell gases nitrogen (1.5 mL min^{-1}) and helium (2.25 mL min^{-1}) were used. The obtained data were processed using

Table 1
Average, minimum and maximum concentrations of detected pesticides in genuine and adulterated oregano.

Compound	Adulterated oregano (34 samples)				Original oregano (42 samples)				All samples (76 samples)			
		Concentration (μg Kg^{-1})				Concentration (μg Kg^{-1})				Concentration (μg Kg^{-1})		
	N	Average	Minimum	Maximum	N	Average	Minimum	Maximum	N	Average	Minimum	Maximum
Acetamiprid	25	6	1	43	23	7	1	52	48	6	1	52
Azinphos-ethyl	0	0	0	0	1	112	112	112	1	112	112	112
Azinphos-methyl	0	0	0	0	1	118	118	118	1	118	118	118
Bifenthrin	0	0	0	0	1	82	82	82	1	82	82	82
Boscalid	0	0	0	0	3	260	16	710	3	260	16	710
Carbendazim	3	164	40	226	3	708	465	900	6	436	40	900
Chlorantraniliprole	0	0	0	0	1	20	20	20	1	20	20	20
Chlorfenvinphos	0	0	0	0	5	31	4	129	5	31	4	129
Chlorothalonil	1	37	37	37	2	185	38	332	3	136	37	332
Chlorpyrifos	34	35	4	187	42	25	4	131	76	29	4	187
Cyfluthrin (sum)	25	167	28	436	1	89	89	89	26	164	28	436
Cypermethrin (sum)	1	57	57	57	5	96	66	138	6	90	57	138
Cyprodinil	2	15	15	15	2	50	16	84	4	33	15	84
Deltamethrin	22	78	44	134	8	69	42	104	30	75	42	134
Difenoconazol	4	20	8	28	7	35	7	115	11	30	7	115
Dimethoate	10	13	5	26	3	19	5	44	13	14	5	44
Dimethomorph (sum)	0	0	0	0	4	10	5	15	4	10	5	15
Diphenylamine	26	14	5	23	28	14	5	20	54	14	5	23
Fenamidone	1	10	10	10	0	0	0	0	1	10	10	10
Fenamiphos (sum)*	1	3	3	3	2	26	16	35	3	18	3	35
Fenthion-sulfoxide	2	7	3	11	0	0	0	0	2	7	3	11
Fipronil	1	20	20	20	0	0	0	0	1	20	20	20
Fluazifop (sum)**	0	0	0	0	6	59	10	116	6	59	10	116
Fludioxonil	4	14	13	15	4	18	16	21	8	16	13	21
Imidacloprid	2	36	6	65	4	12	5	18	6	20	5	65
Indoxacarb (sum)	0	0	0	0	2	63	40	85	2	63	40	85
Iprodione	2	53	38	69	0	0	0	0	2	53	38	69
Lambda-Cyhalothrin	13	79	38	169	1	89	89	89	14	80	38	169
Linuron	0	0	0	0	8	11	1	62	8	11	1	62
Metalaxyl	1	41	41	41	2	41	22	59	3	41	22	59
Methamidophos	1	12	12	12	0	0	0	0	1	12	12	12
Methidathion	16	28	21	38	1	141	141	141	17	35	21	141
Methomyl	1	43	43	43	2	29	26	32	3	34	26	43
Penconazole	1	13	13	13	5	13	9	21	6	13	9	21
Pendimethalin	2	55	48	62	1	46	46	46	3	52	46	62
Permethrin (sum)	14	21	10	53	1	12	12	12	15	20	10	53
Phenothrin (sum)	1	14	14	14	0	0	0	0	1	14	14	14
Phenylphenol, o-	0	0	0	0	2	194	98	290	2	194	98	290
Phosmet	0	0	0	0	1	17	17	17	1	17	17	17
Piperonyl-butoxide	5	20	5	45	6	314	5	1730	11	180	5	1730
Pirimicarb	0	0	0	0	1	34	34	34	1	34	34	34
Pirimiphos-methyl	0	0	0	0	1	29	29	29	1	29	29	29
Profenofos	1	78	78	78	0	0	0	0	1	78	78	78
Propamocarb	1	106	106	106	0	0	0	0	1	106	106	106
Propargite	3	18	10	34	0	0	0	0	3	18	10	34
Propiconazole (sum)	1	23	23	23	0	0	0	0	1	23	23	23
Propyzamide	0	0	0	0	7	98	10	415	7	98	10	415
Pyraclostrobin	1	171	171	171	3	42	15	86	4	74	15	171
Pyrethrins	0	0	0	0	1	446	446	446	1	446	446	446
Pyrimethanil	1	11	11	11	1	30	30	30	2	21	11	30
Pyriproxyfen	26	55	5	153	0	0	0	0	26	55	5	153
Quizalofop (sum)***	0	0	0	0	7	97	5	308	7	97	5	308
Tebuconazole	1	72	72	72	4	2189	56	7310	5	1766	56	7310
Trifloxystrobin	0	0	0	0	1	122	122	122	1	122	122	122
Trifluralin	1	26	26	26	3	15	14	16	4	17	14	26

Fenamiphos (sum)* – Sum of Fenamiphos and Fenamiphos sulfone.
Fluazifop (sum)** – Sum of Fluazifop and Fluazifop-P-butyl.
Quizalofop (sum)*** – Sum of Quizalofop and Quizalofop-P-ethyl.
(sum) – Sum of isomers.

Mass Hunter quantitative analysis software version B.05.02 (Agilent Technologies).

2.5. Liquid chromatography-triple quadrupole mass spectrometry analysis

The analyses of 335 pesticide residues were performed using UHPLC system Acquity Ultra-Performance LC system (Waters, USA). Target pesticides were separated on an Acquity UPLC HSS T3 analytical column (100 mm × 2.1 mm i.d., 1.8 μm particle size, Waters) maintained at 40 °C. 5 mM ammonium formate and 0.1% formic acid in Milli-Q water (A) and 5 mM ammonium formate and 0.1% formic acid in methanol (B) were used for the elution of analytes. The total run time was 22 min. The elution gradient can be briefly summarized as follows: 10–40% B over 4 min, then 40–100% B over 18 min followed by an isocratic hold at 100% B for 4 min. During analysis, the flow rate increased from 0.3 to 0.6 mL min^{-1} in 18 min. Sample volume injected

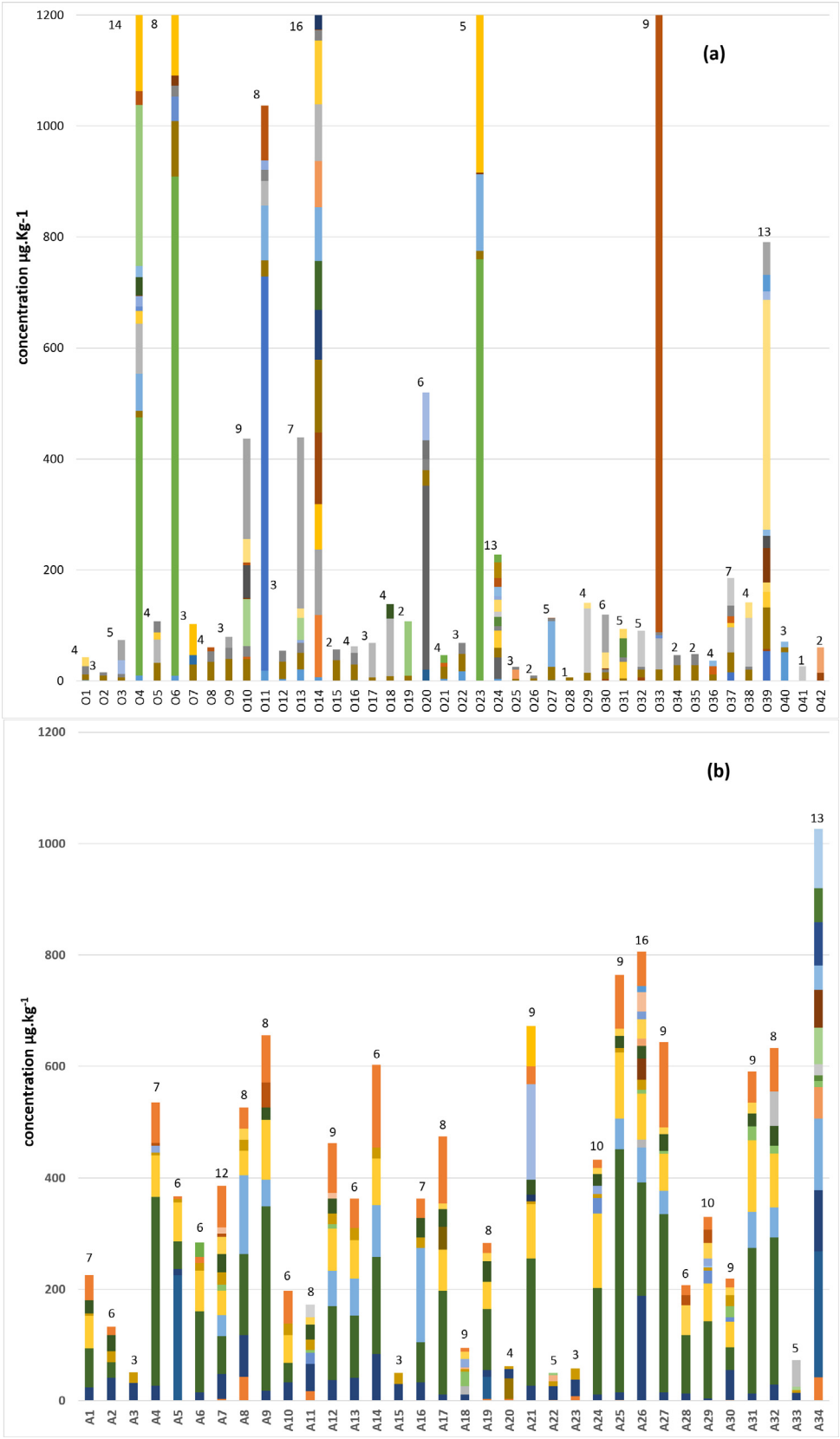

Fig. 1. Total pesticides content (µg Kg^{-1}) in (a) genuine oregano samples and (b) adulterated oregano samples.

was 2.5 μL at 5 °C. The mass spectrometer Xevo TQ-S (Waters, USA) was operated in multiple reaction monitoring (MRM) mode. Electrospray ionization was conducted in positive ion mode (ESI+) with capillary voltage −600 V, ionization and desolvation temperatures were 120 °C and 350 °C, respectively. Nitrogen was used as desolvation and cone gas. The generated data was processed by MassLynx software version 4.1.

3. Results and discussion

3.1. GC-MS/MS and LC-MS/MS validation of the analytical method

As outlined in the Introduction, trace analysis of pesticide residues in oregano, alike in case of other herbs, is a difficult task. Therefore, in the first part of this study, relevant multi-residue methods had to be implemented. To determine a large set of pesticides representing various polarity classes, liquid chromatography and gas chromatography both coupled to triple quadrupole tandem mass spectrometry (GC-QqQ-MS/MS and LC-QqQ-MS/MS) has been used in combination for the determination of pesticide residues potentially co-occurring in oregano samples. The validation of these methods was performed according to the recent EU guidelines SANTE/11813/2017 (2017). At least, two MRM transitions were monitored per compound, the retention time (RT) and corresponding ion ratios were compared with those of matrix-matched calibration standards. The permitted tolerances for the RT were ± 0.1 min for both gas and liquid chromatography; for the relative ion ratio (% of base peak) 30% in MS/MS techniques, in line with this document.

The recovery and repeatability experiments were conducted at two levels, 50 μg kg^{-1} and 200 μg kg^{-1}, with six replicates at each level. The blank matrix used for the experiment was oregano sample from the bio market. The recoveries of the target analytes at both levels ranged between 52 and 120% with RSD values below 21%. The analytes with the recoveries between 52 and 70 % were corrected for the determined recovery. With only a few exceptions (2.5% of target analytes), limits of quantification (LOQs) were ≤50 μg kg^{-1}.

3.2. Analysis of pesticide residues in the set of oregano samples

The oregano samples involved in this study were prior to residue analysis tested for the authenticity applying a two-tier approach using Fourier-Transform Infrared spectroscopy (FTIR) and Liquid Chromatography High Resolution Mass spectrometry (LC-HRMS) developed by Black, Haughey, Chevallier, Galvin-King, and Elliott (2016). Based on those results, the sample set included 34 fraudulent items, containing some form of adulterants such as olive, myrtle, hazelnut, cistus, sumac. The remaining 42 samples were found to be authentic oregano.

The pesticide residues determined in the analyzed samples are summarized in Table S1, where the quantitation limits and the MRLs for each of the detected analytes are also included. The MRLs in this table were calculated for dry oregano (used as a spice) by multiplying MRL set for fresh oregano in the EU pesticides database by´dehydration factor´ proposed by the European Spice Association (ASTA, 2015).

As shown in Table 1 summarizing the generated data, in all of the samples at least one pesticide residue was detected. In most of them (98.5%), multiple residues were present, a cocktail of 16 pesticide species was found in one of the oregano samples. In total, 55 different pesticides were identified. The residue levels ranged from 1 μg kg^{-1} (acetamiprid, linuron) up to 7310 μg kg^{-1} (tebuconazole). Chlorpyrifos, diphenylamine, acetamiprid, deltamethrin, pyriproxyfen, and cyfluthrin were the most frequently found pesticides occurring in 100, 71, 63, 40, 34 and 34% of samples, respectively.

3.3. Genuine oregano

The 42 samples classified as authentic oregano contained between 1 and 16 pesticides per sample, altogether 44 various residues of more than 400 targeted compounds were found (see Fig. 1). Apart from the ubiquitous chlorpyriphos, detected in all the analyzed oreganos, also the residue of diphenylamine and acetamiprid were found frequently, in 67% and 55% of genuine samples, respectively. The other pesticides were detected in less than 50% of these samples. In spite of their high frequency in the analyzed samples, diphenylamine and acetamiprid were detected at low concentrations, ranging from 5 μg kg^{-1} to 20 μg kg^{-1}, and from 1 μg kg^{-1} (LOQ) to 50 μg kg^{-1}, respectively. Slightly higher values were obtained for chlorpyriphos, with concentrations varying from 4 to 131 μg kg^{-1}.

It is important to highlight that some pesticides, although found only in a few samples, occurred at fairly high levels see Table 1, in some cases even exceeding MRLs (see Table S1): tebuconazole in samples O4, O6 and O23; carbendazim in samples O6 and O23 and chlorothalonil in sample O20. In 6 samples also piperonyl butoxide, synergist of synthetic insecticides such as pyrethrins, pyrethroids, rotenone, and/or carbamates, in concentrations ranging from 5 μg kg^{-1} to 1730 μg kg^{-1} was detected.

3.4. Adulterated oregano

The results obtained for the 34 samples of fraudulent oregano showed that all of them contained at least 3 or more pesticides, with 50% of them containing between 6 and 16 residues per sample (see Fig. 1). Similarly to genuine oregano, the most frequently found residues were the following pesticides: chlorpyriphos – Found in all the samples at concentration levels from 4 μg kg^{-1} to 187 μg kg^{-1}, 77% of samples contained following pesticides: acetamiprid – at levels in the range from 1 μg kg^{-1} (LOQ) up to 43 μg kg^{-1}, diphenylamine – at quite low levels in the range of 5 μg kg^{-1} (LOQ)–23 μg kg^{-1} and pyriproxyfen ranged from 55 to 153 μg kg^{-1} The latter compound (registered e.g. as an insecticide for olive trees) was not detected in genuine samples. Several other pesticides such as cyfluthrin, permethrin and deltamethrin occurred mostly in adulterated samples. Of these pyrethroids, cyfluthrin exceeded MRL in 44% of the analysed adulterated oreganos (see Table S1).

3.5. Pesticide residues as adulteration markers

As indicated in the previous results section, notable differences in detected pesticide patterns and their residue levels could be observed between the two groups of samples. This was presumably due to different treatment regimens employed for oregano and for the various plant materials used for adulteration. On this account, in adulterated oreganos, the frequency of detected pesticides was generally higher, the average number of residues per adulterated sample was 7.5 compared to 5.1 in genuine oregano with even more substantial differences found in the medians: 7.5 compared to 4, respectively. While some residues such as acetamiprid, diphenylamine and chlorpyriphos showed comparable detection frequency in both groups of samples, (see Fig. 2), several other pesticides such as lambda-cyhalothrin, deltamethrin, cyfluthrin, permethrin and methidathion had higher occurrence rates in adulterated samples compared to the genuine materials.

To assess differences between sample groups, multidimensional statistical procedures were employed to process generated data; Simca 13.0 software (Umetrics, Sweden) was used for this purpose. Prior to further statistical processing, concentrations of pesticides as determined in oregano samples were normalized (constant row sum) and principal component analysis (PCA) test was performed to exclude outlying samples. A supervised statistical model created then using Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) is shown in Fig. 3. The performance characteristics associated with the resulting

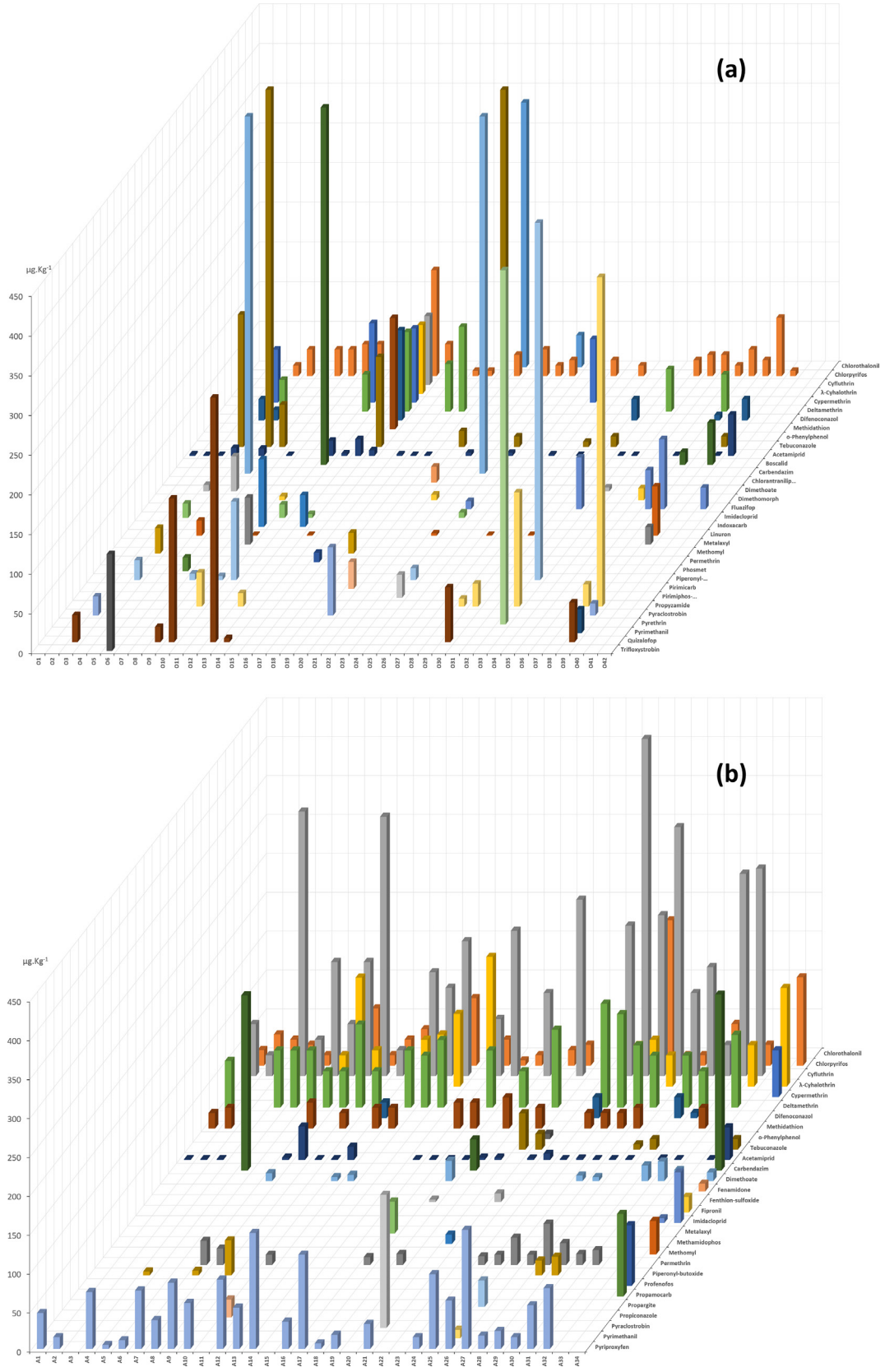

Fig. 2. Occurrence and concentration of target pesticides determined in the analysed oregano samples (a) genuine oregano and (b) adulterated oregano.

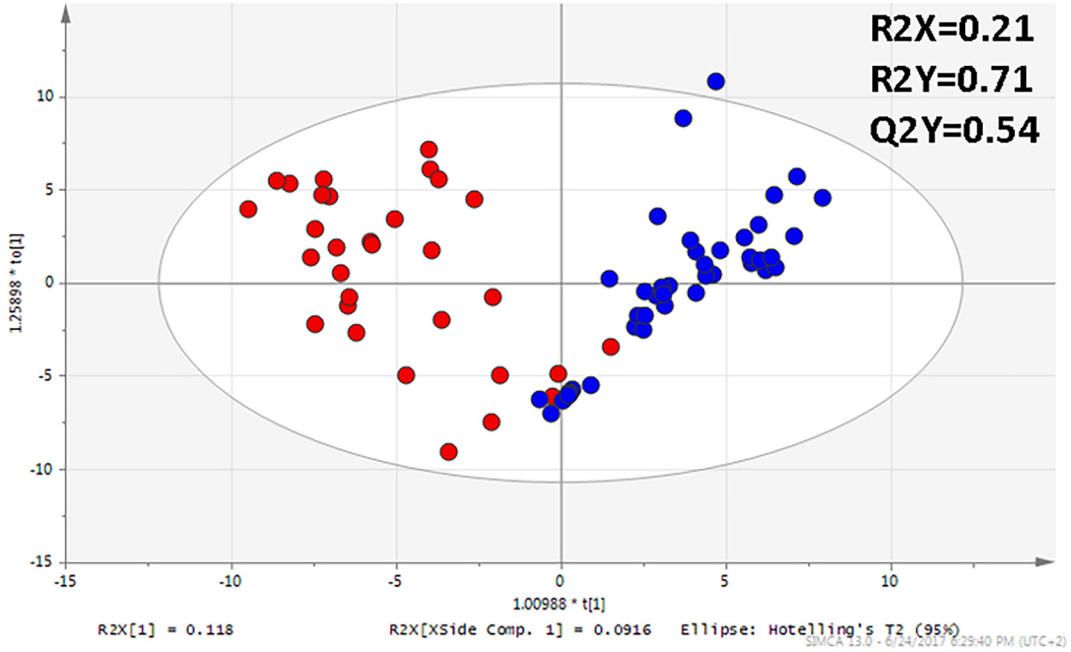

Fig. 3. OPLS-DA scores plot of adulterated and original oregano samples; model based on pesticides relative concentration.

multivariate models were as follows: $R^2(X) = 0.21$; $R^2(Y) = 0.71$ and $Q^2(Y) = 0.54$. The values of R^2 and Q^2 show that good statistical model with a Q^2 value which exceeds acceptable limit 0.5 (Blasco et al., 2015), allowing correct classification of the samples, was obtained.

The S-plot (see Fig. 4) was constructed to identify the most contributing variables which could be the most potentially relevant to identify candidate markers. As shown here, cyfluthrin, pyriproxyfen and cyhalothrin were the most important adulterated oregano markers.

Based on these data, the variable line plot for cyfluthrin, cyhalothrin and pyriproxyfen, showing their normalized concentrations (100% is the sum of the detected pesticides in the analysed sample) (Fig. 5) was outlined. It could be concluded, that any sample, containing more than 10% of cyfluthrin, cyhalothrin lambda or pyriproxyfen, is adulterated.

Moreover, pyriproxyfen was detected exclusively in adulterated oregano samples. It is important to note that this insecticide is not registered for oregano, but is permitted for protection of olive trees leaves, which is the most commonly found adulterant. Under these conditions, its presence at any concentration may indicate oregano adulteration by addition of other plant material. Similarly, the presence of pyrethroids (not detected in genuine oregano) may serve as adulteration markers.

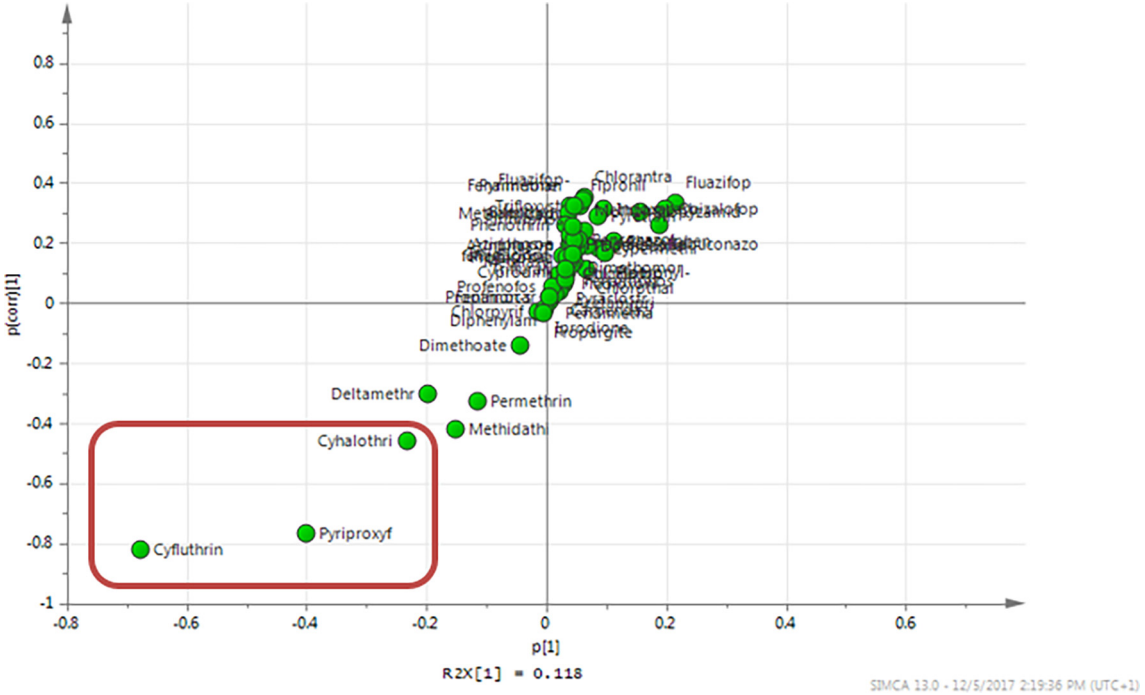

Fig. 4. S-plot of adulterated and genuine oregano samples; model based on pesticides relative concentration.

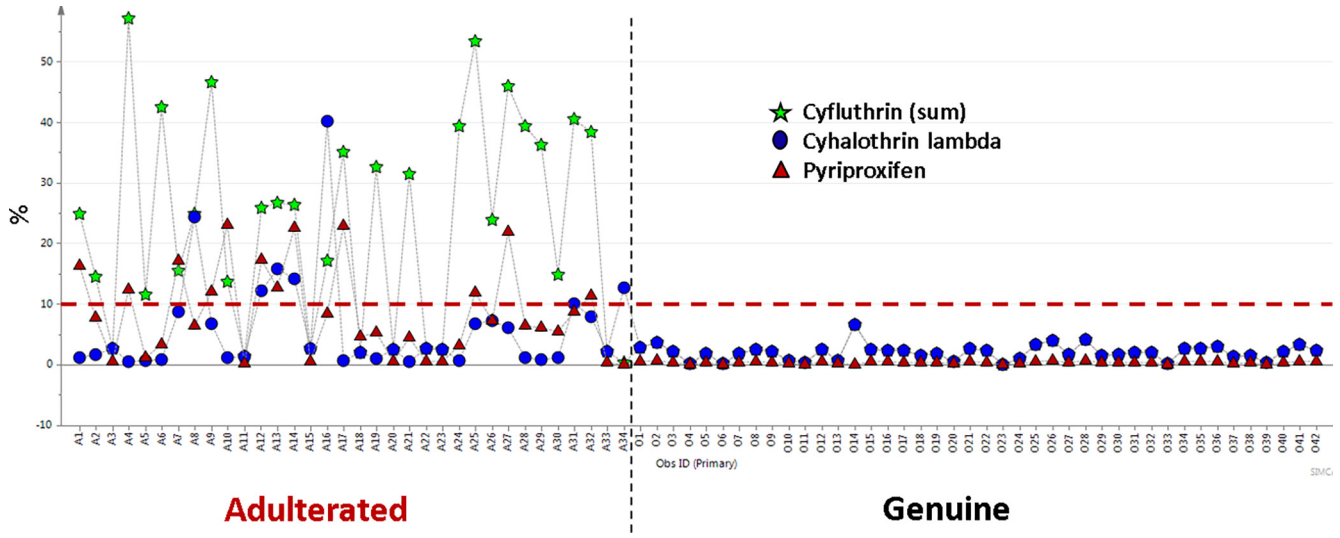

Fig. 5. Variable line plot of selected pesticides. It can be concluded, that the presence of at least of one of these pesticides (at a level higher than 10%) is indicating adulteration.

4. Conclusions

The results obtained in this study can be summarized as follows:

- Altogether 55 different pesticides were identified in the set of 76 oregano samples (34 adulterated and 42 genuine) when using a combination of GC-MS/MS and LC-MS/MS methods covering more than 400 commonly used pesticides.
- Comparing genuine and adulterated samples, a higher number and also higher average concentrations of pesticide residues were found in adulterated samples.
- In the entire set of samples, the most often detected analytes were chlorpyrifos, diphenylamine and acetamiprid, which were found in both, genuine and adulterated samples, at comparable levels.
- The highest concentrations were detected for: tebuconazole: tebuconazole (7310 $\mu g\,kg^{-1}$ and 1050 $\mu g\,kg^{-1}$), carbendazim (900 $\mu g\,kg^{-1}$), and boscalid (710 $\mu g\,kg^{-1}$).
- In 4 genuine samples (~10%) and 15 adulterated samples (44%) the detected concentration of the pesticide residues exceeded the MRL (EU pesticides database, 2017).
- The multidimensional statistical procedure employed for the data assessment showed good performance characteristics: recognition ability $R^2(Y) = 0.71$ and prediction ability $Q^2(Y) = 0.54$; correct classification of adulterated and genuine oreganos was enabled with a high probability.
- Pyriproxyfen, cyfluthrin and cyhalothrin were identified as the most important´markerś of possible adulteration.
- Pyriproxyfen which was not detected in any genuine oregano sample, can be screened for as an oregano adulteration marker.
- Olive leaves, myrtal or other plant materials used as adulterants can, due to different agricultural practices, contain high pesticide residues, moreover, the pattern that is found in such material is different to that found in genuine oregano.
- The purpose of pesticide residues screening in oregano might be not only safety regulataory control but also as a critical assessment of available pesticide data may also enable the identification of suspected adulterated samples. For the confirmation of adulteration complementary analytical approaches should be used.
- Follow-up activities to the present study should be focused on further expanding the database of residues commonly found in authentic and adulterated herb and spice samples. This will allow robust adulteration´markerś to be identified.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2018.09.143.

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