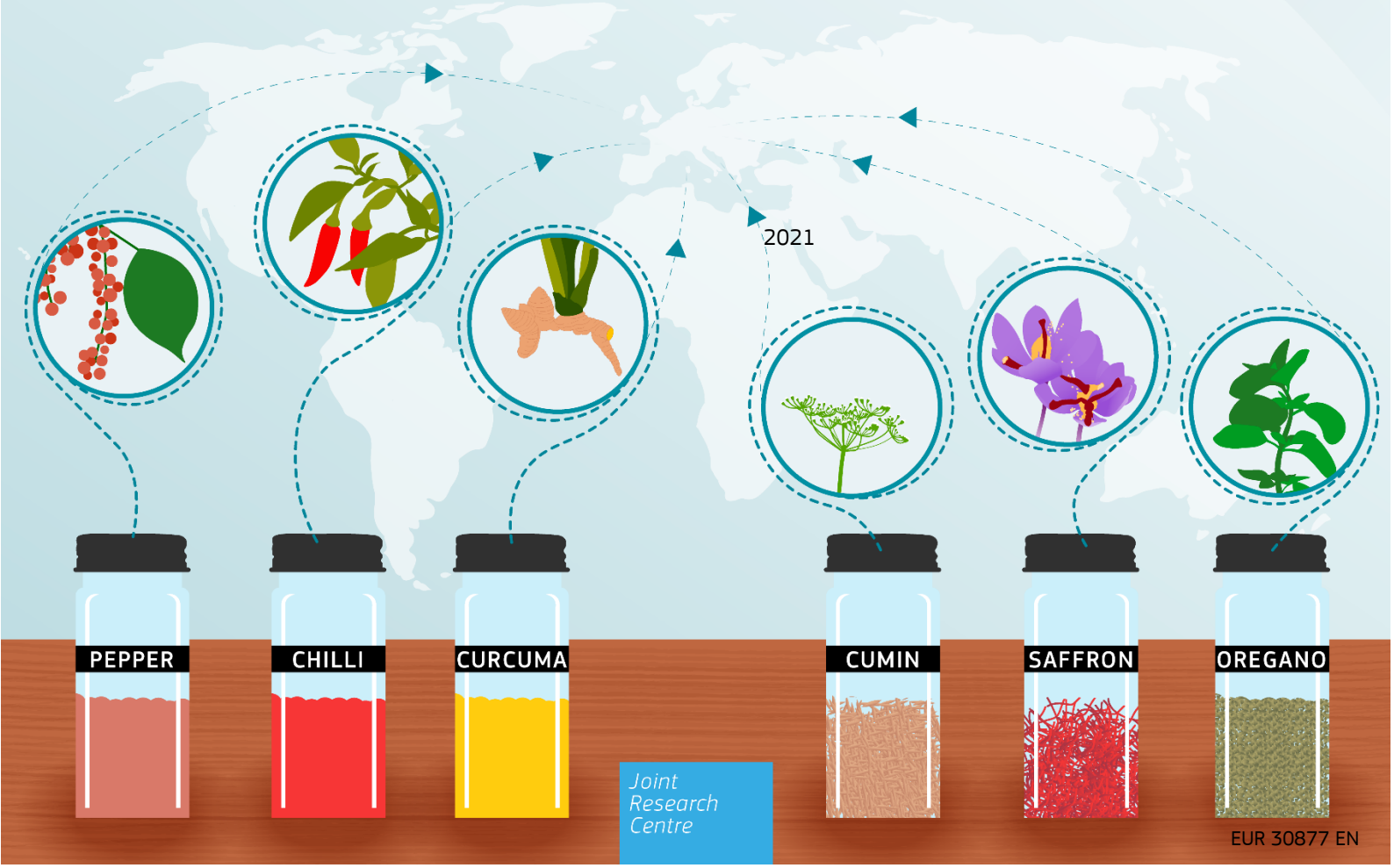
European Commission

JRC TECHNICAL REPORT

Results of an EU wide coordinated control plan to establish the prevalence of fraudulent practices in the marketing of herbs and spices



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Abstract

Culinary herbs and spices are valued for flavouring food and could also provide other beneficial properties, such as antioxidative and bacteriostatic effects as well as certain pharmacological activities. Their supply chain is complex, long, and globalised. Europe is one of the world's leading importing regions for herbs and spices, importing approximately 300.000 tons, mostly spices from East Asia. Most of the spices are produced in countries where certain post-harvest processes such as drying and cleaning may happen before being shipped to the importing country where they are further cleaned and sanitised before being packaged and distributed either to other food businesses or for retail consumption. At each stage, fraudulent manipulations may happen and the more often the material is transferred from one operator to the next, the fraud opportunity increases.

Information available to the European Commission indicates that adulterated herbs and spices are present on the EU market but remain undetected. Therefore, the European Commission set up a coordinated control plan inviting the EU member states to sample certain herbs and spices and send them for analysis to the Joint Research Centre. The main objective of the plan was to establish the prevalence on the market of some non-compliances and of some possible illegal practices into marketing of herbs and spices. Twenty-one EU member states plus Norway and Switzerland submitted nearly 1900 samples to JRC for analysis. The majority of samples was ground or crushed.

The co-ordinated control plan encompassed cumin, curcuma (turmeric), oregano, paprika/chilli, pepper, and saffron, as those were frequently reported to be the target of manipulations.

Nearly 10.000 analyses were carried out on 1885 samples using a range of state-of-the-art analytical techniques to assess the purity of the samples ('true to the name').

The EU coordinated control plan is until now the largest investigation into the authenticity of culinary herbs and spices in terms of participating countries and number of analyses.

The overall rate of suspicious samples was 17% (323 of a total of 1885 analysed samples), which is less than what was previously reported in the scientific literature or by national food control institutions.

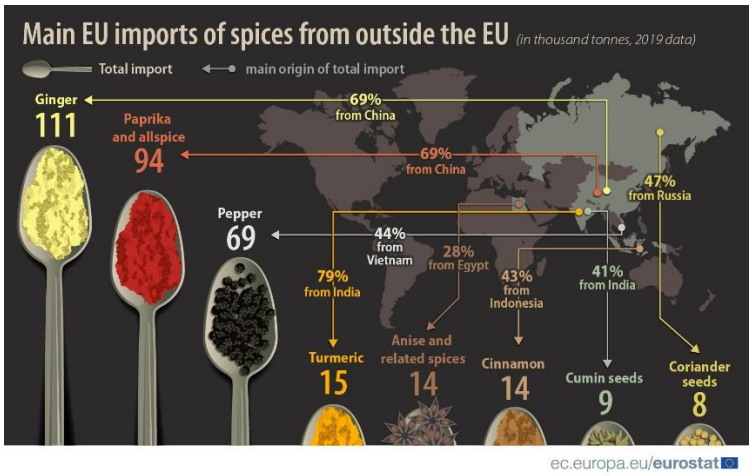
The oregano supply chain was most vulnerable as 48% of samples were suspicious of being adulterated, in most cases with olive leaves. The percentage of samples which were suspicious of adulteration were 17% for pepper, 14% for cumin, 11% for curcuma, and 11% for saffron. The lowest suspicion rate (6%) was found for paprika/chilli. The majority of suspicious samples contained non-declared plant material; in 2% of the analysed spice samples non-authorised dyes were detected. One sample contained a high level of lead chromate.

No specific trend regarding the rate of potential fraudulent manipulations along the supply chain (country of origin/importers/wholesalers/processors/packagers) could be observed. However, the number of samples obtained at certain stages (domestic production, local markets, border control, and internet) was too low to enable statistically meaningful comparisons.

1 Introduction

The EU produces around 100.000 tons of herbs and spices per year, and imports annually over three times this amount, mostly spices, from Asia, Africa, Latin America and the Caribbean; in 2019, the EU Member States imported 379.000 tonnes of spices from non-EU countries (**Figure 1**). The European climate does not allow to grow plant species used for producing spices, except dried paprika and chilli, whereas certain herbs originate from Europe. Intra-EU spice trade consists of re-exports originally coming from non-EU countries.

Figure 1: Main EU imports of spices from outside the EU



The global demand for herbs and spices - and the market for value-added spices and herbs, such as crushed, milled or mixed - is on the rise, with an increasing popularity of the food service sector for their use in ready-made meals, interest in new tastes and ethnic cuisine, health-related claims, etc. The European demand for spices is growing mostly due to interest in new tastes, particularly in East-Asian cuisine, and healthy living. Driven by the desire to have 'clean labels' to better meet the expectations of health conscious consumers, food business operators are keen on using natural ingredients instead of chemically defined additives. The willingness of consumers to pay a premium price for a more natural product has changed the dynamics of various markets and is anticipated to increase the demand for natural herbs and spices (in the cosmetics sector a similar trend to making use of herbs and spices for promoting such products as 'natural' can be observed). Next to their use as seasonings for culinary purposes, herbs and species (or their extracts/essential oils) are the main ingredient for many food supplements and over-the-counter pharmaceuticals. The feed additives sector is also becoming a lucrative market for herbs and spices applications. Given their manifold use, it seems questionable whether the supply will be able to keep up with demand.

Main user of culinary herbs and species is the food processing industry (70-80%), followed by the retail (15-25%) and the food service sector (5-10%).

Supply chains in the herbs and spices sectors tend to be long, complex and can pass through many countries. Often, herbs and spices are farmed at a subsistence scale in non-EU countries and there are frequently many intermediaries in the supply chain offering opportunities for malpractices and/or fraudulent practices.

At consumer level, it may not be feasible to visually identify characteristics of herbs and spices and it may even be totally impossible to identify the plant origin when herbs and spices are crushed or ground.

All these elements generate a high probability for malpractices, some of them with important risks for public health (i.e. substitution of the named herb/spice with an allergenic product and/or colour enhancement by non-authorised dyes).

The herbs and spices supply chain is global, complex, and involves many stages where fraudulent manipulations can happen (**Figure 2**). Vulnerabilities that may affect the chances of adulteration include the length of the supply chain, fraud history, seasonality and availability of the crop, weather events, natural disasters, cultural and geo-political events, economic situation, enforcement of food law, prevalence of corruption, and advances in technology to mask fraud[1]. The drivers of fraud in the herbs and spices markets and the challenges for detecting adulterants have been reviewed by various authors[2],[3],[4].

Figure 2. Vulnerability of the herbs and spices supply chain.

[1] British Retail Consortium | Food and Drink Federation| Seasoning and Spice Association: Guidance on authenticity of herbs and spices. Industry best practice on assessing and protecting culinary dried herbs and spices. https://www.fdf.org.uk/globalassets/resources/publications/guidance-herbsandspices.pdf

[2] Galvin-King, P., Haughey, S.A., Elliott, C.T. Food Control 88 (2018) 85-97

[3] Silvis, I.C.J., van Ruth, S.M., van der Fels-Klerx, H.J., Luning, P.A. Food Control 81 (2017) 80-87

[4] Osman, A.G., Raman, V., Haider, S., Ali, Z., Chittiboyina, A.G., Khan, I.A. Journal of AOAC International 102 (2019 2) 376-385

Agri-Food fraud is about "any suspected intentional action by businesses or individuals for the purpose of deceiving purchasers and gaining undue advantage therefrom, in violation of the rules referred to in Article 1(2) of Regulation (EU) 2017/625 (the agri-food chain legislation)"[5]. In the herbs and spice sector, fraudulent manipulations include but are not restricted to[6]:

- ingredients, additives, dyes or any other constituent not approved for use in food and/or herbs and spices;
- ingredients, additives, dyes or any other constituent approved for use in food but unlawfully not declared or indicated in a form which might mislead the customer;
- spices or herbs that has had any valuable constituent omitted or removed which misleads the customer (e.g. spent and partially spent spices and herbs, de-oiled material, defatted material);
- a different part of the same botanical plant, rather than the one declared to an extent that this is misleading the customer;
- technically avoidable amounts of parts from other botanical plants than the one declared.

Spices were mentioned in the European Parliament resolution of 14 January 2014 on the food crisis, fraud in the food chain and the control thereof[7], among the commodities that are most vulnerable to fraud. An inventory made by researchers from Wageningen University and Research places herbs and spices at the top of 9 products most vulnerable to adulteration[8].

French authorities (Direction générale de la concurrence, de la consommation et de la répression des fraudes) investigated in 2019 anomalies in the domestic spice market and found irregularities in 26.4% of the 138 samples (cumin, curcuma, paprika/chilli, oregano, pepper, saffron). In an earlier investigation, carried out in 2016, the suspicion rate was 50%[9].

This information signals that herbs and spices not meeting the requirements laid down on the basis of Regulation (EC) No 178/2002 are present on the EU market. Malpractices remain largely undetected, particularly because many EU Member States have rather limited control activities related to the authenticity of herbs and spices. Consequently, and in line with the Council Conclusions of 16 December 2019[10], calling upon the European Commission to continue with coordinated control plans on detecting and investigating food fraud, a coordinated control plan was designed to establish the prevalence of non-compliances and illegal practices in the marketing of herbs and spices in the European Union plus Norway and Switzerland.

This report summarises the outcome of the coordinated control plan.

[5] https://ec.europa.eu/food/safety/agri-food-fraud/food-fraud-what-does-it-mean_en

[6] European Spice Association: ESA Adulteration Awareness Document. https://www.esa-spices.org/index-esa.html/publications-esa

[7] European Parliament resolution of 14 January 2014 on the food crisis, fraud in the food chain and the control thereof (2013/2091(INI)). https://www.europarl.europa.eu/doceo/document/TA-7-2014-0011_EN.html

[8] Weesepoel en Van Ruth (2015): Inventarisatie van voedselfraude: mondiaal kwetsbare productgroepen en ontwikkeling van analytische methoden in Europees onderzoek. Wageningen, RIKILT Wageningen. https://www.wur.nl/upload_mm/8/b/8/600b715e-fb64-4a89-868e-e0fc0bb4072d_Rapport%202015.014_LR.pdf

[9] https://www.economie.gouv.fr/files/files/directions_services/dgccrf/presse/communique/2021/CP-Epices.pdf

[10] Council of the European Union, General Secretariat of the Council: Council conclusions on the next steps how to better tackle and deter fraudulent practices in the agrifood chain (15154/19). https://www.consilium.europa.eu/media/41865/st15154-en19.pdf

2 Coordinated Control Plan

The objective of this coordinated control plan (CCP) was to establish the prevalence of some non-compliances and illegal practices in the marketing of herbs and spices in the European Economic Area.

The competent authorities were asked to implement this coordinated control plan by verifying traceability and labelling of selected herbs and spices through documentary and physical checks, including sampling for laboratory analysis, which were carried out by the Joint Research Centre.

The sampling plan prioritised the earliest possible control points of the food chain (80% of the samples should be taken at border control posts, producers, importers and wholesalers, storage/processing/packaging establishments) over the end of the chain (20% of the samples at distribution and retail level).

The sampling strategy targeted presentation of herbs and spices which are more susceptible to fraudulent practices as outlined previously in this document (i.e.: crushed, milled and ground products).

The testing activities focussed on the detection of a (partial) substitution of the named herb/spice by another botanical material, the extension by addition of fillers (e.g. starch, flour, dust, chalk, etc.) and/or the enhancement of colour by a non-authorised additive (e.g. synthetic dye).

Other fraud types such as misdescription of origin or agricultural production system (conventional/ organic) or conservation treatment (ionizing radiation) were not part of the CCP.

2.1 Herbs and spices included in the coordinated control plan

Herbs and spices are a very diverse group of products; therefore, a selection had to be made taking into account their commercial value and history of fraud cases. The following six were included in the CCP:

Cumin (*Cuminum cyminum*)

Fraud history includes the presence of mahaleb, a species closely related to almonds, but the source of this contamination remained unclear. Other reported bulking agents are peanut shells and almond husks.

Curcuma (*Curcuma longa*)

Widely used as a spice (as part of curry powder) but also for Ayurvedic medicine. Fraud history includes illegal colour enhancement with azo-dyes but also with inorganic materials (yellow chalk, lead chromate) and extension with fillers (maize or rice flour, etc.).

Oregano (*Origanum vulgare*)

Typical herb of the Mediterranean, which is in high demand. Fraud history includes partial substitution with olive leaves, sumac and myrtle.

Paprika/chilli (*Capsicum annuum*)

High import volume and high economic value. Fraud history includes illegal colour enhancement with azo-dyes and extension with tomato skins.

Pepper (*Piper nigrum*)

Highest import volume among spices in EU and high economic value. Fraud history includes substitution of whole peppercorns as well as ground pepper by papaya seeds and extension of ground pepper with fillers.

Saffron (*Crocus sativus*)

Most expensive spice and frequently adulterated by colour enhancement with azo-dyes and substitution with other botanicals (e.g. safflower, turmeric, other *Crocus* spp.).

2.2 Participation in the coordinated control plan

Twenty-one EU Member States plus Norway and Switzerland submitted 1900 samples for analysis. Fifteen samples were outside the scope of the CCP (spice blend, spice extract, wrongly attributed) and were therefore not analysed. **Table 1** and **Annex 1** summarises per country the number of analysed samples, broken down into cumin, curcuma, oregano, pepper, paprika/chilli, and saffron.

Table 1. Herbs and spice samples included in the coordinated control plan.

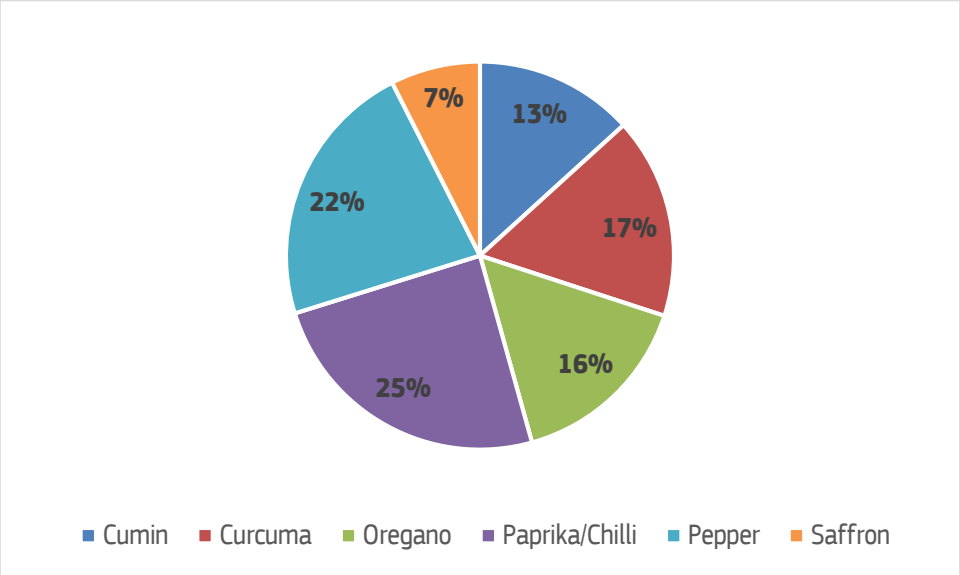
	Number of samples						
	Analysed	Cumin	Curcuma	Oregano	Paprika/Chilli	Pepper	Saffron
Austria	70	4	12	14	15	13	12
Belgium	98	19	20	15	17	21	6
Croatia	70	12	13	14	15	11	5
Cyprus	20	4	5	2	4	5	0
Denmark	95	19	15	11	30	19	1
France	141	24	28	25	28	23	13
Germany	156	15	26	25	47	36	7
Greece	90	17	16	18	19	15	5
Hungary	95	14	16	16	31	17	1
Ireland	63	11	10	10	11	12	9
Italy	99	10	21	16	19	27	6
Latvia	54	7	9	6	14	17	1
Lithuania	54	10	10	6	15	9	4
Luxembourg	48	7	10	5	10	11	5
Malta	20	3	3	2	7	3	2
Norway	16	0	0	8	8	0	0
Poland	93	9	19	20	25	18	2
Portugal	111	19	20	11	29	30	2
Romania	119	4	18	19	34	44	0
Slovenia	50	1	8	7	15	16	3
Spain	143	15	10	19	35	29	35
Sweden	87	12	12	13	17	27	6
Switzerland	93	14	15	13	17	18	16
TOTAL	1885	250	316	295	462	421	141

Distribution of the types of herbs and spices in the total sample collection was well balanced; only saffron, which is an expensive but less widely used spice, was represented to a lesser extent (**Figure 3**). The majority of the samples (89%) were processed, i.e. ground or crushed.

2.3 Laboratory testing

An overview of the applied analytical methods for detecting (i) non-authorised dyes, (ii) inorganic fillers, and (iii) undeclared botanicals is given in **Figure 4**. Details of the methods are provided in Annex 2.

Figure 3: Percentage distribution of herbs and spices in the sample panel of the coordinated control plan



2.4 Compliance assessment

Samples that contained colours (dyes) not approved for use in herbs and spices and listed in Annex II of Regulation (EC) No 1333/2008[11] and/or not respecting the maximum residue limit (MRL) of certain elements set in Annex II of Regulation (EC) No 396/2005[12] were considered as non-compliant.

As specific provisions for authenticity and purity of herbs and spices do not exist in the EU regulatory framework, besides the requirements of the General Food Law (Article 8)[13] and the Food Information to Consumers (Article 7)[14] regulations, the relevant ISO standards, in particular the provisions for extraneous matter and total ash, formed the basis for assessing purity of herbs and spices:

ISO 959-2:1998 Pepper (*Piper nigrum* L.), whole or ground -- Specification -- Part 2: White pepper

ISO 7540:2006 Ground paprika (*Capsicum annuum* L.) – Specification

ISO 972:1997 Chillies and capsicums, whole or ground (powdered) – Specification

ISO 3632-1:2011 Spices -- Saffron (*Crocus sativus* L.) -- Part 1: Specification

ISO 7925:1999 Dried oregano (*Origanum vulgare* L.) -- Whole or ground leaves – Specification

ISO 5562:1983 Turmeric, whole or ground (powdered) -- Specification

ISO 6465:2009 Spices -- Cumin (*Cuminum cyminum* L.) – Specification

The ISO standard for the determination of extraneous matter and foreign matter content (ISO 927:2009) provides for a visual examination of the material, which was found inappropriate as

[11] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives

[12] Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC

[13] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety

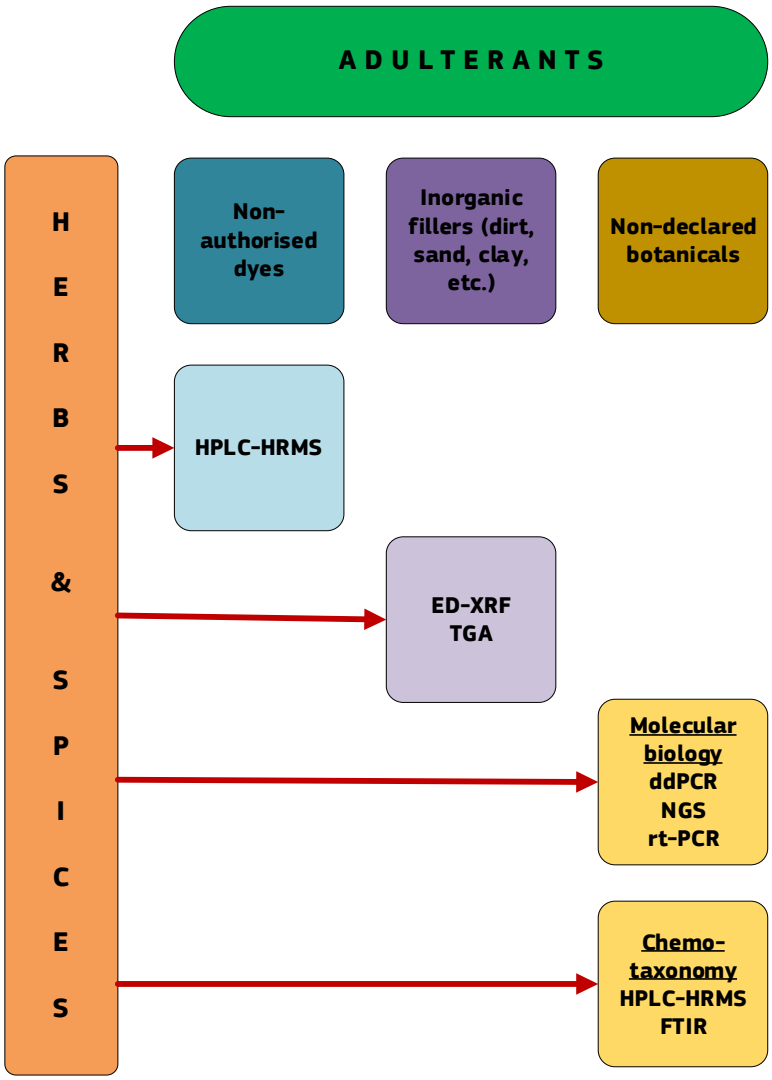
[14] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004

most of the samples were either crushed or ground. Therefore, DNA based testing was used instead to determine the presence of extraneous matter.

In case a sample did not comply with the ISO provisions for extraneous matter and total ash, it was considered to be suspicious of adulteration. In addition, the outcome of additional tests targeting certain biomarkers of herbs and spices was used as supporting evidence.

Detailed decision rules for compliance assessment of the herbs and spices included in the CCP are given in Annex 3.

Figure 4: Analytical methods to detect adulterants in herbs and spices.



HPLC-HRMS, High performance liquid chromatography coupled to high resolution mass spectrometry; ED-XRF, Energy dispersive X-ray fluorescence spectroscopy; TGA, thermogravimetric analysis; ddPCR, Digital droplet polymerase chain reaction; NGS, Next generation sequencing; rt-PCR, Real-time PCR; FTIR, Fourier transform infrared spectroscopy.

3 Results

In total, 9926 analyses were carried out by the JRC in the frame of the CCP on 1885 samples; 1340 samples were analysed by high performance liquid chromatography coupled to high resolution mass spectrometry (HPLC-HRMS) to detect the presence of non-authorised dyes, 1885 samples were analysed by digital droplet PCR (ddPCR) to screen them for possible presence of non-declared botanicals, 1460 samples were submitted to meta-barcoding by next generation sequencing (NGS) to create an overview of species present in a sample, and 647 species-specific, real time-PCR (rt-PCR) assays were done to confirm the presence of a non-declared plant species and approximate its amount. In addition, 1885 samples were analysed by energy dispersive-X-ray fluorescence and by Fourier transform infrared spectroscopy (FTIR), 697 by HPLC-HRMS and 127 by thermogravimetric analysis (TGA) to provide chemistry based evidence to support decision making.

3.1 Presence of non-authorised colours (dyes)

The overall adulteration rate of spices with non-authorised dyes was 2% (25 samples out of 1340).

All curcuma, paprika/chilli, pepper, and saffron samples submitted by the Member States were checked by HPLC-HRMS for the presence of non-authorised synthetic dyes (**Table 3**).

In 316 curcuma samples, Sudan I was present in one sample, Tartrazine in two samples. All paprika/chilli samples analysed contained the main chemical markers (capsanthin, capsaicin, dihydrocapsaicin, nordihydrocapsaicin); in 10 samples the following non-authorised dyes were detected: Sudan I in one sample, Allura Red in six samples, Bixin in 7 samples, Azorubin in two samples and Sunset Yellow in one sample. None of the pepper samples contained non-authorized dyes and the main chemical marker compound piperine was present in all samples. Out of 141 saffron samples, 12 contained the following unauthorized dyes: Sudan I in one sample, Sunset Yellow in four samples, Azorubine in two samples, Acid Yellow 3 in two samples, Tartrazine in seven samples, Carminic acid in two samples, Allura Red in one sample and Auramine O in three samples (some samples contained more than one dye).

Table 2: Presence of non-authorised synthetic dyes in herbs and spices.

	Number of samples		
	Analysed	Containing non-authorised dyes	% Samples containing non-authorised dyes
Curcuma	316	3	0.9
Paprika/chilli	462	10	2.2
Pepper	421	0	0.0
Saffron	141	12	8.5

In one curcuma sample substantial amounts of lead (2 g/kg) and chromium (0.5 g/kg), presumably in the form of lead chromate, was found by ED-XRF analysis. Lead chromate has been reported as an adulterant to enhance the bright yellow colour of curcuma.

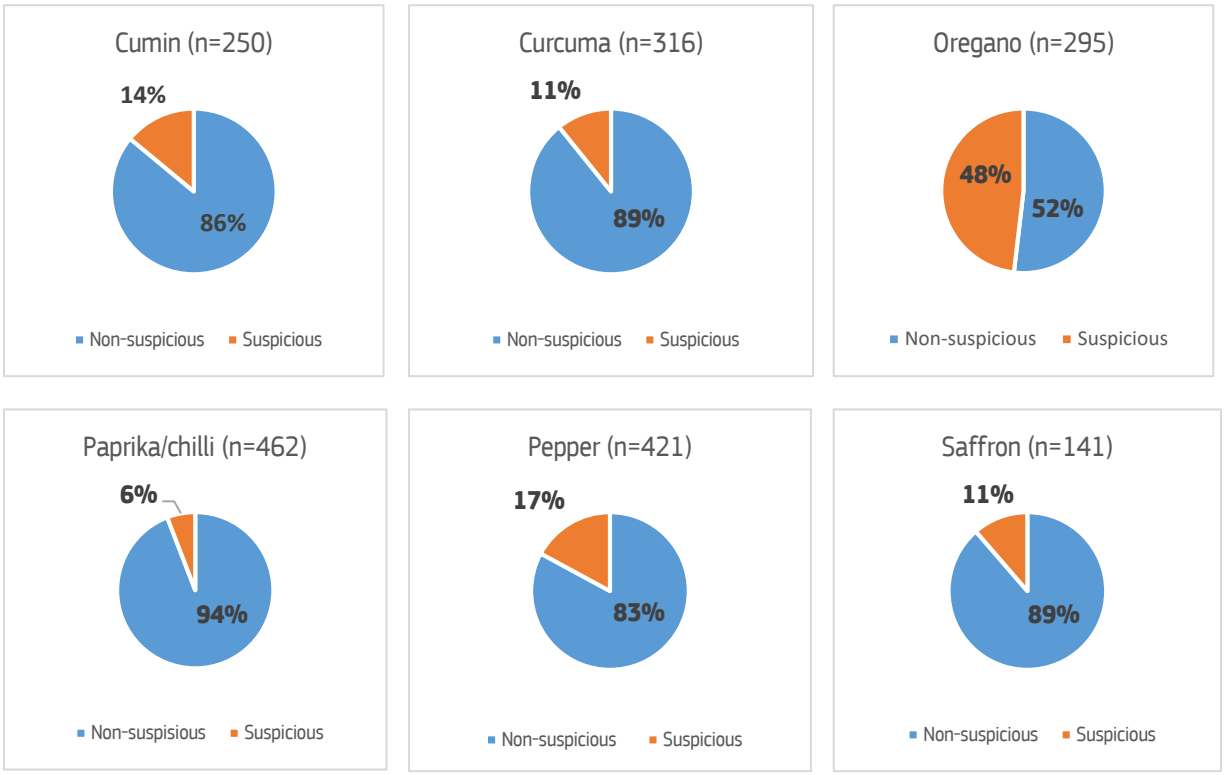
3.2 Authenticity of herbs and spices

Assessing the authenticity of culinary herbs and spices goes beyond the question whether the named species is present but tries to answer how much of the named species makes up the sample; in other words how 'pure' the sample is. A combination of DNA-based methods, i.e. ddPCR, meta-barcoding by NGS and rt-PCR, and chemistry-based methods, i.e. fingerprinting by HPLC-

HRMS, FT-IR and ED-XRF, and ash determination by TGA, were used to decide whether a sample is suspicious of adulteration.

The overall rate of suspicious samples was 17% across all samples included in the CCP; **Figure 5** provides a breakdown according to the spice/herb type. A high proportion of oregano samples (48%) were found to be suspicious of adulteration, whereas for cumin, curcuma, pepper, and saffron the proportion of suspicious samples was between 10% and 20%. For paprika/chilli the proportion was less than 10%.

Figure 5: Proportion of herbs and spices samples found suspicious of adulteration. Four cumin samples submitted by Member States were in fact 'caraway', correctly labelled in the vernacular language, and one sample of curcuma was a curcuma extract; those samples were excluded from the statistics.



3.2.1 Cumin

In some Member States the vernacular name under which 'cumin' is sold is in fact caraway (*Carum carvi*), whereas in other Member States *Cuminum cyminum* is meant. This can lead to confusion if the scientific name of the spice is not given on the label or accompanying document.

Four of the 254 samples received were labelled as caraway (confirmed by DNA analysis) and were, therefore, excluded from the statistical analysis.

Of the 250 remaining samples, 34 (14%) were suspicious of being adulterated. Seven samples contained a substantial amount of caraway (*Carum carvi*) although labelled as cumin (*Cuminum cyminum*). This finding was supported by the presence of carvone, a chemical substance which is typical for caraway.

Twelve samples contained coriander (*Coriandrum sativum*), three mustard (*Sinapis alba*), one linseed (*Linum* spp.) and another sample pumpkin seed (*Cucurbita pepo*) above the maximum level of extraneous substances. Ten samples had a total ash content above the maximum level set by ISO 6465:2009.

In nine samples DNA of mustard (*Brassica* spp./*Sinapis alba*), which is a food allergen, was detected.

The majority of samples were collected from importers/wholesalers/processors/packagers. Highest rates of suspicious samples were observed at the point of entry into the internal market and with samples collected at local markets. However, the number of samples taken at those points was too low to make statistically meaningful comparisons (**Table 4**).

Table 3: Distribution of tested samples along the cumin supply chain.

	Number of samples			
Source	Total	Non-suspicious	Suspicious	% Suspicious
Domestic Producer	1	1	0	0
Local market	10	9	1	20
Border	7	5	2	29
Importer / Wholesaler	92	78	14	15
Processor / Packager	69	63	6	9
Retailer	67	56	11	16
Internet	4	4	0	0
Total	250	216	34	14

3.2.2 Curcuma

Thirty-four (11%) of the 316 curcuma (turmeric) samples tested were suspicious of being adulterated; one sample was in fact an extract of curcuma and was not included in the statistical evaluation. Three of the thirty-four suspicious samples contained non-authorised dyes (one contained Sudan I, two samples Tartrazine), six samples contained less than 2% curcuminoids as required by the ISO specification (ISO 5562:1983), and in 24 samples DNA of non-declared plant material, mostly paprika/chilli (*Capsicum* spp.) and starch containing species such as maize (*Zea mays*), rice (*Oryza sativa*), and other cereals (*Avena* spp./*Triticum* spp.) were detected in amounts greater than 2%, which is the maximum allowed amount of extraneous material in curcuma according to ISO 5562:1983. One sample behaved unusually as it contained only curcumin and none of the other naturally present curcuminoids (demethoxycurcumin and bisdemethoxycurcumin) and none of the DNA tests detected *Curcuma* DNA. Therefore, the sample was declared suspicious.

The majority of samples were collected from importers/wholesalers/processors/packagers. The rate of suspicion increased slightly from 11% at border inspection posts to 15% at the processor/packager stage. However, the number of samples taken at those points was too low to make statistically meaningful comparisons (**Table 4**).

Table 4: Distribution of tested samples along the curcuma supply chain.

	Number of samples			
Source	Total	Non-suspicious	Suspicious	% Suspicious
Domestic Producer	3	2	1	33
Local market	10	9	1	10
Border	9	8	1	11
Importer / Wholesaler	118	108	10	9
Processor / Packager	104	88	16	15

Retailer	66	61	5	8
Internet	4	4	0	0
Unknown	2	2	0	0
Total	316	282	34	11

3.2.3 Oregano

One hundred forty-two (48%) of the 295 oregano samples tested were suspicious of being adulterated. In 80 samples olive (*Olea europaea*) DNA was found, which was supported by the presence of the chemical marker oleuropein, a substance specific for *Olea europaea*, in all those samples.

Origanum majorana, which shall not be present in dried oregano according to the ISO standard, was present in 11 samples, myrtle (*Myrtus communis*) in three samples, and a starch containing filler in one sample.

Most samples contained low levels of thyme (*Thymus vulgaris*), peppermint (*Mentha x piperita*) and sage (*Salvia* spp.) DNA. *Origanum* belongs to the same tribe (Mentheae) within the Lamiaceae subfamily Nepetoideae as *Mentha*, *Thymus* and *Salvia* and because of this close phylogenetic relationship, a low resolution in species and genus attribution to the DNA reads is expected for some of the used barcodes. However, as for 47 samples a higher number of thyme (*Thymus vulgaris*), peppermint (*Mentha x piperita*) and sage (*Salvia* spp.) DNA reads were found, they were considered suspicious of adulteration.

The majority of samples were collected from importers/wholesalers/processors/packagers. Samples taken at the point of entry into the internal market and from e-commerce had a high rate of suspicious samples, but the number of samples taken at those points was too low to make statistically meaningful comparisons (**Table 5**).

Table 5: Distribution of tested samples along the oregano supply.

	Number of samples			
Source	Total	Non-suspicious	Suspicious	% Suspicious
Domestic Producer	3	1	2	67
Local market	13	4	9	69
Border	11	1	10	91
Importer / Wholesaler	109	60	49	45
Processor / Packager	97	54	43	44
Retailer	55	32	23	42
Internet	6	1	5	83
Unknown	1	0	1	100
Total	295	153	142	49

3.2.4 Paprika/Chilli

Twenty-seven (6%) of the 462 paprika/chilli samples tested were suspicious of being adulterated. One sample tested positive for a number of other species but after closer inspection of the label it

turned out to be a paprika containing spice mix, which was therefore excluded from the statistical evaluation.

In 10 of the 27 suspicious samples non-authorised dyes were detected (cf. 3.1), five samples had a total ash content higher than 10% (mass/mass), which is the maximum level set by the ISO 927:1997 specification, and the remaining suspicious samples contained non-declared plant species. In most instances maize (*Zea mays*), carrot (*Daucus carota*), tomato (*Solanum lycopersicum*), sunflower seed (*Helianthus annuus*), and *Allium* spp. (most likely onion or garlic) was found in excess of 1% (maximum level according to ISO 927:1997).

The majority of samples were collected from importers/wholesalers/processors/packagers. However, samples taken at different points in the supply chain had a similar rate of suspicious samples, with a tendency to a higher proportion at the samples taken at the border and at the premises of processors/packagers (**Table 6**).

Table 6: Distribution of tested samples along the paprika/chilli supply chain**.**

	Number of samples			
Source	Total	Non-suspicious	Suspicious	% Suspicious
Domestic Producer	14	13	1	7
Local market	35	34	1	3
Border	13	12	1	8
Importer / Wholesaler	171	160	11	6
Processor / Packager	132	122	10	8
Retailer	96	93	3	3
Internet	1	1	0	0
Total	461	434	27	6

3.2.5 Pepper

Seventy (17%) of the 421 pepper samples tested were suspicious of being adulterated with non-declared plant material.

Thirty-seven (9%) contained starch containing fillers such as rice (*Oryza sativa*), buckwheat (*Fagopyrum esculentum*), and other cereals (wheat - *Triticum* spp., barley - *Hordeum vulgare*), nine contained mustard seed (*Brassica* spp. and/or *Sinapis alba*), which is an allergen. Fourteen samples did not comply with the minimum content for piperine (4% m/m) – in 10 of them DNA of non-declared plant material was found as well – and six samples were above the maximum content of 7% (m/m) total ash set by the ISO specifications. The remaining samples tested positive for non-declared other spices such as paprika (*Capsicum annuum*), garlic (*Allium sativum*), cumin (*Cuminum cyminum*), fennel (*Foeniculum vulgare*), coriander (*Coriandrum sativum*) above a level of 2.5%, the maximum for extraneous matter set by the ISO specification.

The majority of samples were collected from importers/wholesalers/processors/packagers; a comparatively lower number of samples were taken at the entry into the internal market, where the rate of suspicious samples was lower in relation to other sampling points along the value chain. Contamination due to cross-contact could be the reason for the higher rates observed at the processing stage (**Table 7**).

Table 7: Distribution of tested samples along the pepper supply chain.

	Number of samples			
Source	Total	Non-suspicious	Suspicious	% Suspicious
Domestic Producer	2	1	1	50
Local market	25	21	4	16
Border	26	23	3	12
Importer / Wholesaler	139	115	24	17
Processor / Packager	121	99	22	19
Retailer	104	88	16	17
Internet	4	4	0	0
Total	421	351	70	17

3.2.6 Saffron

Sixteen (11%) of 141 samples tested were suspicious of being adulterated. Four samples consisted mainly of safflower (*Carthamus tinctorius*) and one of marigold (*Tagetes* spp.). In four of the five samples the saffron-specific compounds crocin and safranal were absent, which strengthens the suspicion that they were adulterated. In addition, in one of those samples a non-authorised dye (Sudan I) was found. Non-authorised dyes were also present in 11 samples that did not contain extraneous plant material (some of them contained multiple dyes).

The majority of samples were collected from importers/wholesalers/processors/packagers, which had a comparatively low rate of suspicious samples, whereas samples taken from local markets and purchased over the Internet had much higher rates. Samples taken at the point of entry into the internal market and from e-commerce had a high rate of suspicious samples but the number of samples taken at those points was too low to make statistically meaningful comparisons (**Table 8**).

Table 8: Distribution of tested samples along the saffron supply chain.

Source	Total	Non-suspicious	Suspicious	% Suspicious
Domestic Producer	4	4	0	0
Local market	9	4	5	56
Border	12	10	2	17
Importer / Wholesaler	47	44	3	6
Processor / Packager	37	35	2	5
Retailer	30	27	3	10
Internet	2	1	1	50
Total	141	125	16	11

4 Conclusions

Culinary herbs and spices are a globally traded commodity with a complex supply chain, including many steps before reaching the end users, i.e. the food and hospitality industry and consumers. Owned to the complexity of the chain, manifold opportunities exist to adulterate culinary herbs and spices to improve economic gain.

The coordinated control plan used analytical testing of samples taken along the supply chain to determine the prevalence of some potential non-compliances and illegal practices in the marketing of herbs and spices in the European Economic Area. Another aspect was to identify at which stage of the chain fraudulent manipulations are more frequently observed. Food inspection authorities and food business operators can profit from this knowledge as it will allow to better target control activities and strengthen preventive measures to combat food fraud.

- The coordinated control plan is until now the largest investigation into the authenticity of culinary herbs and spices in terms of participating countries (21 EU Member States plus Norway and Switzerland) and samples analysed (1885).
- A broad set of state-of-the-art analytical tools was used to assess the purity of the samples ('true to the name'). Rules to decide whether a sample is suspicious of adulteration considered the outcome of several complementary techniques. However, the techniques were not designed to grade the quality of the assessed herbs and spices.
- The overall rate of suspicious samples was 17% (323 of a total of 1885 analysed samples), which is less than what was previously reported in the scientific literature or by national food control institutions.
- The oregano supply chain was most vulnerable as 48% of samples were suspicious of being adulterated, in most cases with olive leaves.
- The percentage of samples which were suspicious of adulteration were 17% for pepper, 14% for cumin, 11% for curcuma, and 11% for saffron.
- The lowest suspicion rate (6%) was found for paprika/chilli.
- The majority of suspicious samples contained non-declared plant material; in 2% of the analysed spice samples non-authorised dyes were detected. One sample contained a high level of lead chromate.
- In two cumin, 45 oregano, and four pepper samples copper compounds above the relevant maximum residue limit set by Regulation (EC) No 396/2005 were found.
- No specific trend regarding the rate of potential fraudulent manipulations along the supply chain (countries of origin/importers/wholesalers/processors/packagers) could be observed. However, for certain stages (domestic production, local markets, border control, and internet) the number of samples tested was too low to enable statistically meaningful comparisons.

List of abbreviations and definitions

ddPCR	Digital droplet polymerase chain reaction
ED-XRF	Energy dispersive X-ray fluorescence spectroscopy
FTIR	Fourier transform infrared spectroscopy
HPLC-HRMS	High performance liquid chromatography coupled to high resolution mass spectrometry
ISO	International Standardization Organization
NGS	Next generation sequencing
rt-PCR	Real-time polymerase chain reaction
PCA	Principal component analysis
TGA	Thermogravimetric analysis

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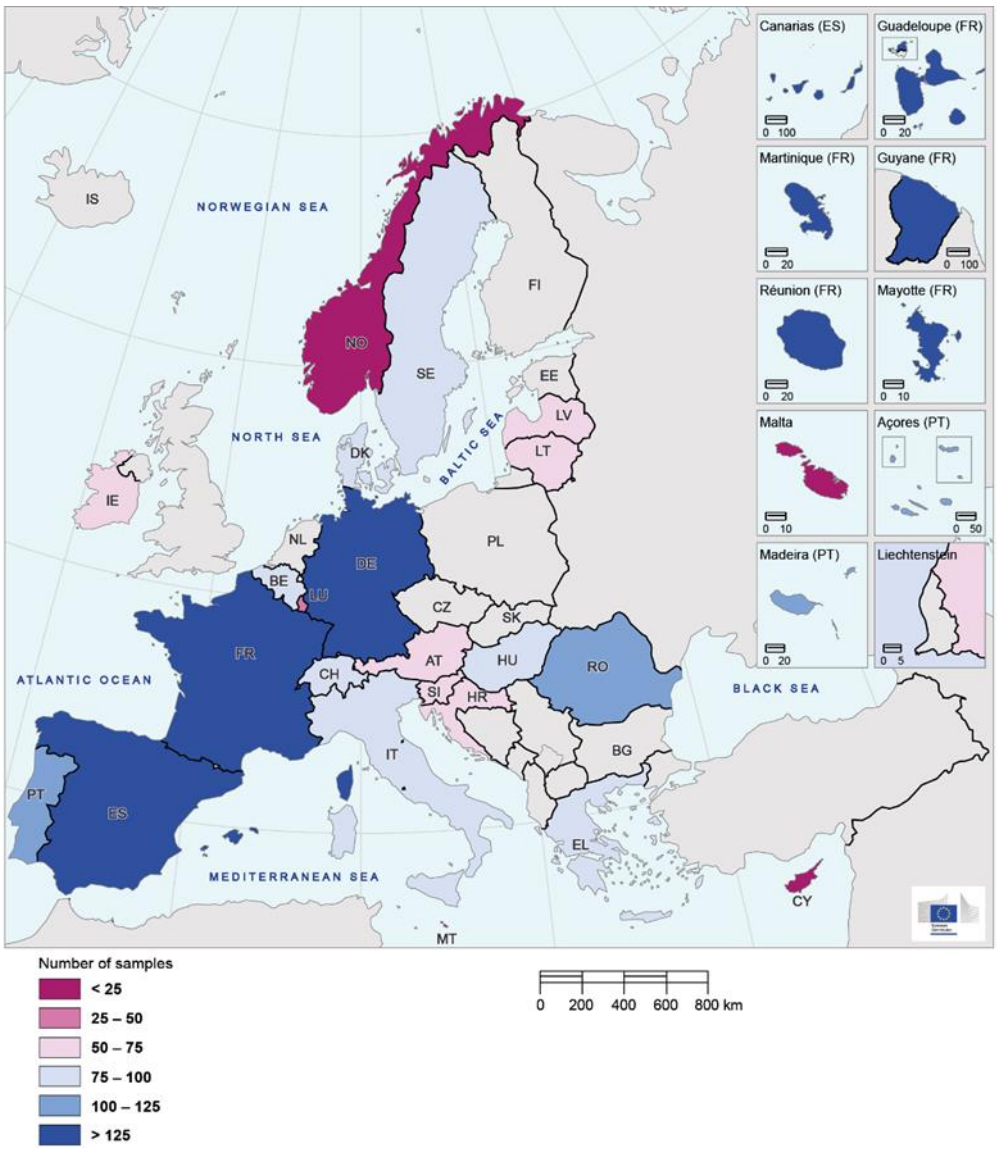
Annexes

Annex 1. Participation of EEA countries in the coordinated control plan



Annex 2. Analytical methods applied to identify suspicious samples in the frame of the coordinated control plan for herbs and spices

A1.1 High performance liquid chromatography – high resolution mass spectrometry

An UltiMate™ 3000 high performance liquid chromatograph coupled to a Q Exactive™ mass spectrometer (Thermo Fisher) together with an Acquity BEH C18 analytical column and a gradient from 5% to 95% of 0.1% formic acid in water to 0.1% formic acid in acetonitrile was used for the quantitative determination of the following non-authorized dyes:

Sudan I, Sudan II, Sudan III, Sudan IV, Sudan Red B, Sudan Orange G, Sudan red G, Sudan Red 7B, Butter Yellow, Sunset Yellow FCF, Para Red, Bixin, Rhodamine B, Orange II, Metanil Yellow, Toluidine Red, Azorubin, Fast Garnet GBC base, Chrysoidine G, Tropaeolin O, Fast Green FCF, Tartrazine, Allura Red AC, Amaranth, Indigo carmine, Erythrosin Yellowish, Brilliant Blue FCF, Oil Orange SS, Auramine O, Ponceau 4R, Ponceau6R, Sudan Black B, Acid Yellow 3, Yellow 2G, Red 2G, Carminic acid, Sulforhodamine B, Acid Red 73, Astaxanthin, Naphthol Yellow S, Fluorescein sodium, Crystal Violet, Patent Blue V calcium, Green S, Citrus Red 2, Ponceau 3R, Malachite Green, in pepper, paprika/chilli, saffron and curcuma samples.

Additionally, the method was applied to evaluate the presence of the following main characteristic chemical markers: piperine in pepper; capsanthin, capsaicin, dihydrocapsaicin, nordihydrocapsaicin in paprika/chilli; crocin, safranal in saffron; curcumin, demethoxycurcumin, bisdemethoxycurcumin in curcuma (turmeric).

Untargeted analysis was done with the same HPLC-HRMS system. Data was processed by using the XCMS package of R, which was employed for automatic pre-processing of all metabolic fingerprints, filtering, grouping, retention time correction, re-grouping, filling missing data and normalisation, resulting in a features data set. A quality control (QC) sample was prepared by pooling aliquots of each sample, which was included in the run sequence. The XCMS output was further subjected to Principal Component Analysis (PCA) using SIMCA-P software. Samples outside the 95% confidence interval of the PCA plot were considered to be outliers and the obtained data were used as supporting evidence for deciding whether a sample is suspicious.

A1.2 Energy dispersive – X-ray fluorescence spectroscopy

An Epsilon 5 (PANalytical, Almelo, The Netherlands) ED-XRF spectrometer was used to determine the concentrations of Mg, Al, Si, P, Cl, S, K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, Br, Rb, Sr, Zr, Cd, Ba, Pb and Hg.

Samples were pulverised in a planetary mill and around 6 g powder was used to prepare 40 mm diameter pellets that were then analysed by X-ray fluorescence.

SIMCA Version 15.0.2 (Sartorius Stedim Biotech AS, Malmö, Sweden) software was used to exploit the information content of the whole element profile provided by ED-XRF analysis. The PCA score plot served to test the existence of clusters and to gather information about the elements that contributed mostly to the formation of those clusters.

A1.3 Thermogravimetric analysis (TGA)

The ash content of the pepper test materials was determined with a ThermoGravimetric Analyzer (Mettler Toledo). Test materials were measured in duplicate with test portion sizes ranging from 35 to 64 mg. The test portions were heated in an oxidative atmosphere to 550 °C and held at that temperature until the weight of the residue stayed constant. The weight % of the residue was reported as the ash content.

A1.4 Digital droplet PCR (ddPCR)

Digital droplet PCR was used to estimate the number of target copies, which was then related to the expected number of DNA copies calculated from the genome weight of the target species. A prediction interval around the regression line (i.e. the range of measurements in which 95% of all future observations are expected to lie, provided the samples are of comparable purity as the reference sample) was established by analysis of samples of known purity and compatibility to market samples. Samples falling within the prediction interval were considered as compliant and those falling outside as suspicious.

Automated DNA extraction was performed using a Tecan Freedom EVO liquid handler with Promega chemicals (CTAB extraction buffer, CLD lysis buffer, Reliaprep Resin, BWA wash buffer) and the Promega Purefood protocol.

Fluorometric DNA quantification was done on a Qubit 4 (Invitrogen) with High Sensitivity chemistry (Invitrogen) according to manufacturer instructions. The following primer pairs were used in ddPCR assays:

Species	**Molecular target**	**Primer sequences**
Cumin *(Cuminum cyminum)* developed in house	Limonene synthase	TCGAAACGCTACATGGTGGA GTTATACTCACCAGTCCATTGC
Curcuma *(Curcuma longa)* developed in house	Chalcone synthase	CATCGAAGGGGTCGAGAAT GCAGACCGTTCTCCTTCAAC
Oregano *(Origanum vulgare)* Agliassa et al, 2018	Elongation factor 1	CTCCAGTTCTTGATTGCCACAC GCTCCTTTCCAGACCTCCTATC
Paprika/chilli *(Capsicum annuum/C. frutescens)* developed in house	Pungency locus *pun1*	CATCCTCATGCATCTCTTGC GAGAGCAACCATCACCAATC
Pepper *(Piper nigrum)* *Hao* et al, 2016	Hydroxycinnamoyl transferase	GCCGCAGATTCTCAAGGA CGAAGTCGCCGAAGTCAT
Saffron *(Crocus sativus)* developed in house	Mg-protoporphyrin monomethyl ester cyclase (putative)	GAACTGGTGTCAGGATGAGA GGCCATGAATTAATGATGCAA

Primer pairs were tested for cross-reactivity with other species by rt-PCR using SYBR green chemistry.

The materials employed for method development and validation were either prepared from fresh and dry materials commercially available through shops and garden centres (where possible, single plants/fruits were used). Plant and/or DNA reference materials were obtained from the Meise Botanical Garden (Nieuwelaan 38, 1860 Meise, Belgium), the Kew DNA bank (The Royal Botanic Gardens, Kew, DNA Bank) and from the DNA Bank of the Botanic Garden and Botanical Museum Berlin (BGBM). All DNA samples as well as underlying voucher specimens are deposited at the Botanic Garden and Botanical Museum Berlin (BGBM) and are available via the Global Genome Biodiversity Network (GGBN) (Droege et al, 2014) and the Global Biodiversity Information Facility (GBIF). Plant material and DNA were provided under the agreement of the Convention on Biological Diversity (1992).

Digital PCR reactions were performed using the Biorad QX200 digital droplet platform. Reactions were set up using Evagreen Supermix (Biorad).

A1.5 Sequencing and meta-barcoding

Extracted DNA was amplified by PCR to generate the five barcodes (i.e. *RbcL*, *TrnL*, *psbA*, *MatK*, ITS) recommended by the Consortium for the Barcode of Life (CBOL) Plant Working group. Since the five

barcodes have different annealing temperatures, five separated PCR reactions were performed. The amplification products were then purified using either the QIAquick PCR Purification Kit, Qiagen, or magnetic beads (Mag-Bind® TotalPure NGS, Omega BIO-TEK) and quantified (Qubit).

DNA barcode libraries were prepared using the Ion Plus Fragment Library Kit (ThermoFisher), following the manufacturer's recommendations. Subsequently, the libraries were pooled in an equimolar amount into the template reaction for attachment of the fragments to Ion Sphere Particles (ISP) and clonal amplification in emulsion-PCR. The template reaction was conducted on the Ion OneTouch 2 instrument (ThermoFisher). Enriched samples were subsequently sequenced on the Ion GeneStudio S5 System (ThermoFisher), using the Ion 520 chip, which produced 3-5 million reads (1-2 Gb).

The sequencing data obtained were analysed on the Torrent Suite Software and then with a custom-tailored software for species identification, provided by ThermoFisher. The software clustered all the reads and then performed a BLAST against the NCBI nt database (downloaded locally), providing the number of reads attributed to a species with a certain degree of identity (by default higher than 99%). The results were then analysed to evaluate how many reads are attributed to the species of interest, and how many reads belong instead to non-declared species.

A1.6 Real-time PCR (rt-PCR)

Real time-PCR was used to confirm the presence of adulterants and contaminants found by NGS, and, if applicable, to semi-quantify their amount. All rt-PCR reactions were amplified in ABI microamp 96-well 0.1 ml Fast plates using an Applied Biosystems QuantStudio S7 (Life Technologies). The following molecular markers were used for targeting the species:

Species	**Molecular target**	**Primer sequences**
Achiote (*Bixa orellana*) Marieschi et al, 2012	SCAR marker	ACTTTTCAAAGCCGACACGC ATCTGGACAATAGCTTTAACGC
Almond (*Prunus* spp.) Burns et al, 2016	Internal transcribed spacer ITS2	TAGCAGAACGACCCGAGAACTAG CGCCGGTGTTCGTTTGTAC
Barley (*Hordeum vulgare*) Hernandez et al, 2005	γ-Hordein	AGACAAGGCGTGCAGATCG GACCCTGGACGAGCACACAT
Bindweed (*Convolvulus arvensis*) developed in house	phi1 homospermidine synthase	CCCGGTCTAATCGTTGACAT CAAGGATAAGCGCTCCAGTC
Black caraway (*Bunium persicum*) developed in house	3-Hydroxy-3-methylglutaryl coenzyme A reductase	TTCTTCATGTGATTTCCCCG ATTTTCCACGCCCCTCAATC
Buckwheat (*Fagopyrum esculentum*) developed in house	Allergenic protein AF216801.1	CGCCAAGGACCACGAACAGAAG ATCGCATTTCGCTACGTTCTTCATCG
Brassica spp. Mbongolo Mbella et al, 2011	Cruciferin	CAGCTCAACAGTTTCCAAACGA CGACCAGCCTCAGCCTTAAG
Brassica species distinction Koh et al, 2017 Genome A	SSR and SNP markers	GTTTTGGCCGTAAATCCCAC GTTACGGGTAGCGTGTGTC
Genome B		GGCATCTGAAGAGAGAGTC

		CCATCTTCTTCTTGCCATG
Genome C		TGCTGCGCCGAACAATAG CCGATCGTGGTTCATATTGC
Caraway (*Carum carvi*) developed in house	contig 222854 MarkerID Cc2019M026	GGCTGGAACTTTTTATTCAC TGAGGGAAAACCAGGATGGA
Carrot (*Daucus carota*) developed in house	Aspartokinase-homoserine dehydrogenase	GCAGAGATAGTTGTGGAGGA CGAGCGCAGATTCATAAGAA
Chickpea (*Cicer arietinum*) Nakamura et al, 2018	9-cis-Epoxycarotenoid dioxygenase	ATCAGCCACAACAGCATCAAAC TTTAAGCTCAAATCTTTGAAAGGAG
Coriander (*Coriandrum sativum*) developed in house	Delta-4-palmitoyl-ACP desaturase	CAGTGCCCAAAAAGGAACAT CTGACAGTGGGCTAGCATGA
Cumin (*Cuminum cyminum*) developed in house	Limonene synthase	TCGAAACGCTACATGGTGGA GTTATACTCACCAGTCCATTGC
Curcuma (*Curcuma longa*) developed in house	Chalcone synthase	CATCGAAGGGGTCGAGAAT GCAGACCGTTCTCCTTCAAC
Fennel (*Foeniculum vulgare*) developed in house	Glyceraldehyde 3-phosphate dehydrogenase	CCCCTCTTTTTGGTCTGCAT CAGCTCTTCCACCTCTCCAG
Fenugreek (*Trigonella foenum-graecum*) developed in house	Centromere CenH3	CCAGATACGACACTGACACGTA CAAACCTATGTCGGTGTCTGA
Garlic (*Allium sativum*) developed in house	Alliinase	GCCTCATTACAGCCCAATCA CATCCTTTATCAACGCCCAC
Goosefoot (*Chenopodium album*) developed in house	Phosphoenolpyruvate carboxylase-1E1	AGGACTACCACTGAATCTGC CTCCAAATCCAAGCCACACA
Kava (*Piper methysticum*) Jiang et al, 2009	SCAR marker	GGTCACCTCAAACCAAGCTTAATCAAG GGTCACCTCATAATACAAACTTGCAAGC
Linseed (*Linum* spp.) developed in house	Cellulose synthase	GCTGTAATGATCGGTGGTTC GGGAAACTTATCTTGATCGTC
Maize (*Zea mays*) EURL MON-87460-4 method	High Mobility Group protein	TTGGACTAGAAATCTCGTGCTGA GCTACATAGGGAGCCTTGTCCT
Marjoram (*Origanum majorana*) Focke et al, 2011	rDNA ITS region	AACCTCGAAAAGTAGACTGTGA TCGATCCCCCAAACACGC
Morning glory (*Ipomoea* spp.) Park et al, 2018	Inverted repeat region	CATCCATGGCTGAGTGGTGA CTATGCGCGGGTTCAATTCC
Mustard, white (*Sinapis alba*) Fuchs et al, 2010	MADS D	TGAAAACTCTCTTCCCCTCTTAGG ACAAATGCACACAAGACAGAGATATAGA
Myrtle (*Myrtus communis*) developed in house	Retrotransposon Ty1-copia-like element Tmc1	TTCGAAATACCCGTTATGGAAA GTGCCCGAATCCGAAGATTG
Oats (*Avena* spp.)	3-Phosphoglycerate kinase	GATATCTCTCCTGTAGGCTG

developed in house		CCACTCCCACCTTCTCAACA
Olive (*Olea europaea*) developed in house	Stearoyl-acyl carrier protein desaturase	ATGAGAAGCGCCATGAAACT CTTCCGACCAAAAATTCCAA
Oregano (*Origanum vulgare*) Agliassa et al, 2018	Elongation factor 1	CTCCAGTTCTTGATTGCCACAC GCTCCTTTCCAGACCTCCTATC
Papaya (*Carica papaya*) Wei et al, 2016	Chimopapain	CCATGCGGATCCTCCCA CATCGTAGCCATTGTAACACTAGCTAA
Paprika/chilli (*Capsicum* spp.) developed in house	Pungency locus *PUN*	CATCCTCATGCATCTCTTGC GAGAGCAACCATCACCAATC
Peanut (*Arachis hypogaea*) Scaravelli et al, 2008	2S Albumin	GAACCAGAGCGATAGGTTGC CGCCATTTCGACTTCCAA
Pepper (*Piper longum*) developed in house	Internal transcribed spacer ITS2	GTCTGGTCGTCCGTGTGCT AACGCGCTGACAATCGTG
Pumpkin (*Cucurbita pepo*) developed in house	Aspartic acid proteinase inhibitor	CTTGATCTTGGCTGGTGTAG GTTTGGCAGAGATAATGAGG
Radish (*Raphanus sativus*) developed in house	Anthocyanidin reductase	TCGTGCCCATCTGTTTCTTG ATTTCTGTGGGAGCTAAATG
Rice (*Oryza sativa*) EURL LLRICE601 method	Phospholipase D	TGGTGAGCGTTTTGCAGTCT CTGATCCACTAGCAGGAGGTCC
Safflower (*Carthamus tinctorius*) Marieschi et al, 2012	SCAR marker	ACAACCATTGGAGATTCCGG AGTGAGCACTCTTAGTTAACC
Starch-producing poaceae species developed in house	Granule-bound starch synthase 1	ATGATGTTGTCGAGCTCGC AGATCAACTGGATGAAGGCC
Sunflower (*Helianthus annuus*) Hernandez et al, 2005	11S Storage protein G3-D1	CTCGAGCACCTCCGGCT GCCCTGCAAGGTTTGCTATC
Tomato (*Solanum lycopersicum*) Focke et al, 2011	ITS (Internal transcribed spacer)	GACCCGCGAACTCGTTTTA TTAACAGAGCAGCGCGCTT
Wheat (*Triticum aestivum*) Iida et al, 2005	Waxy-D1	GTCGCGGGAACAGAGGTGT GGTGTTCCTCCATTGCGAAA

Primer pairs were tested for cross-reactivity with other species by rt-PCR using SYBR green chemistry.

Wherever required the amount of non-declared plant species identified by NGS was based on the number of genome copies and not as weight-weight percentage and should, therefore, be regarded as semi-quantitative. Although discrepancies may exist between copy-% and weight-%, a large body of experience from the field of GMO quantification exists that shows these differences tend to be small. In practice, a calibration curve was made with a pure reference material for which the number of haploid genome copies was regressed against the PCR signal (Cq value). The copy number in each reaction was calculated based on (i) the amount of DNA (as measured by fluorometry), and (ii) the haploid genome weight (as retrieved from the KEW plant 1C-value database[15]). The number of spice genome copies present in a sample was determined using the

[15] https://cvalues.science.kew.org/

same principle to obtain a percentage for the adulterant/contaminant (# adulterant copies / # spice copies).

A1.7 Determination of marker compounds by high performance liquid chromatography – high-resolution mass spectrometry (LC-HRMS)

Pepper was extracted with ethanol using a modified version of ISO 11027:1993[16]. After dilution of the extract, it was injected into a HPLC-HRMS system (Orbitrap Elite, Thermo Scientific) with an Agilent Eclipse Plus C18 50 x 2.1 mm, 1.8 µm column at 400 µL/min. Detection was done in positive mode electro spray ionisation with single MS full scans in the range from m/z 50 to m/z 500 to quantify the content of piperine (%) by comparison to a reference solution of pure piperine.

Curcuma was extracted with methanol using a modified version of AOAC 2016.16[17]. Curcuminoids (sum of curcumin, demethoxycurcumin, bisdemethoxycurcumin), were separated using a Kinetex PFP 50 x 2.1 mm, 1.7 µm column at 600 µL/min and detected by the high-resolution mass spectrometer with an APCI source in positive mode between m/z 50 and 2000. The same system was used to determine the presence of biomarkers indicating the presence of cumin (cumin aldehyde), paprika/chilli (capsaicin) and pepper (piperine).

A1.8 Attenuated Total Reflectance – Fourier Transform Infrared (ATR-FTIR) spectroscopy

FTIR spectroscopy was performed in the spectral range of 400-4000 cm^{-1} by using a Vertex spectrometer from Bruker (Germany) equipped with a platinum-ATR. Spectra were pre-processed by Standard Normal Variate (SNV) and dimensionality reduction was done by principal component analysis (PCA) using Unscrambler®X v10.5 (CAMO Software, Trondheim, Norway). PCA score plots were used to visualize patterns in the distribution of the pepper samples, and the loading plots to interpret the spectral information when necessary. Samples outside of the 95% confidence interval of the PCA plot were considered to be outliers.

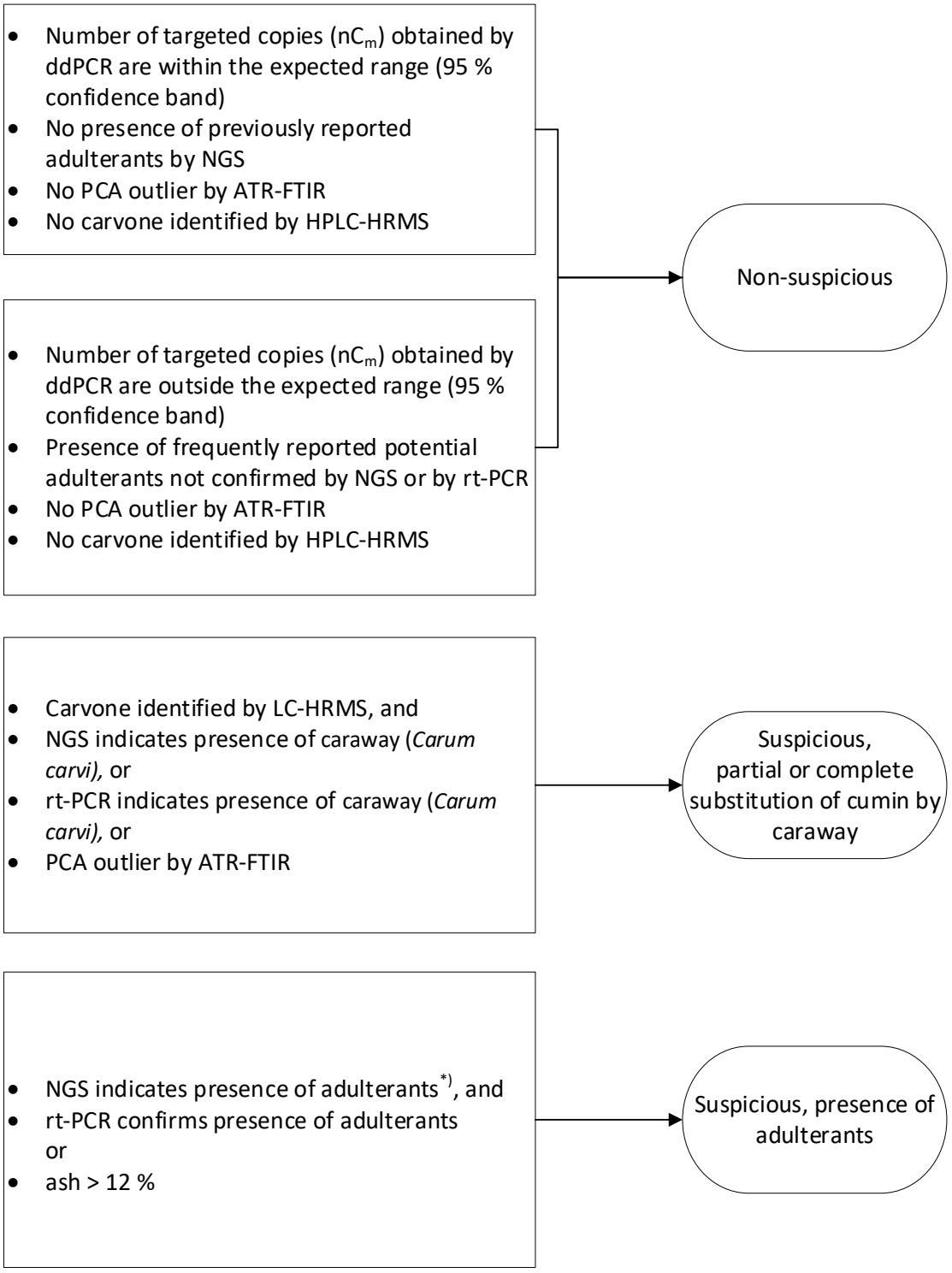
N.B. *JRC's quality system is ISO 9001 certified and certain testing activities of JRC Geel are ISO 17025:2017 accredited. However, the methods of analysis used for generating the reported data are outside the scope of accreditation but all of them were single-laboratory validated.*

[16] ISO 11027:1993 Pepper and pepper oleoresins – Determination of piperine content – Method using high performance liquid chromatography

[17] AOAC Official Method 2016.16 Curcuminoids in Turmeric Raw Materials and Dietary Supplements

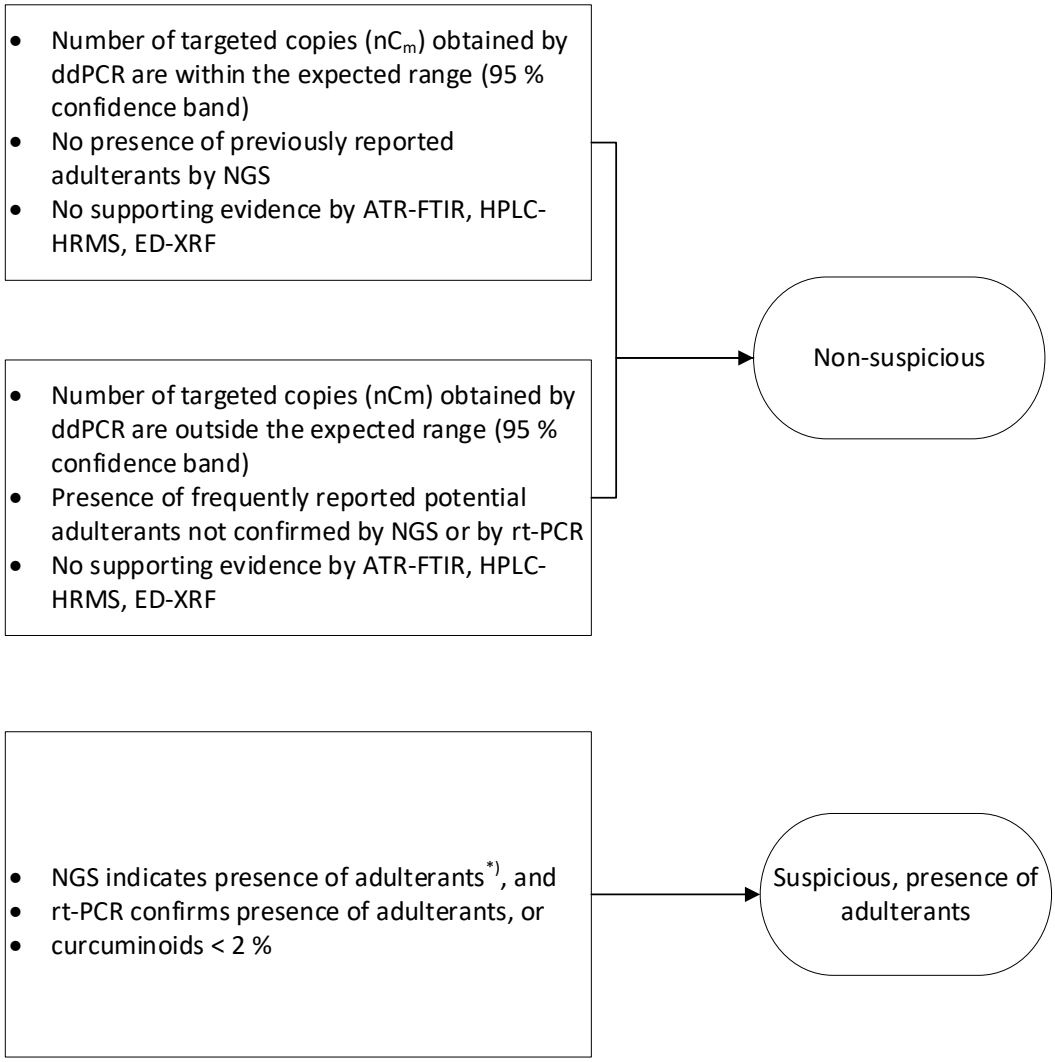
Annex 3. Decision rules to assess authenticity of herbs and spices

Cumin (*Cuminum cyminum*)



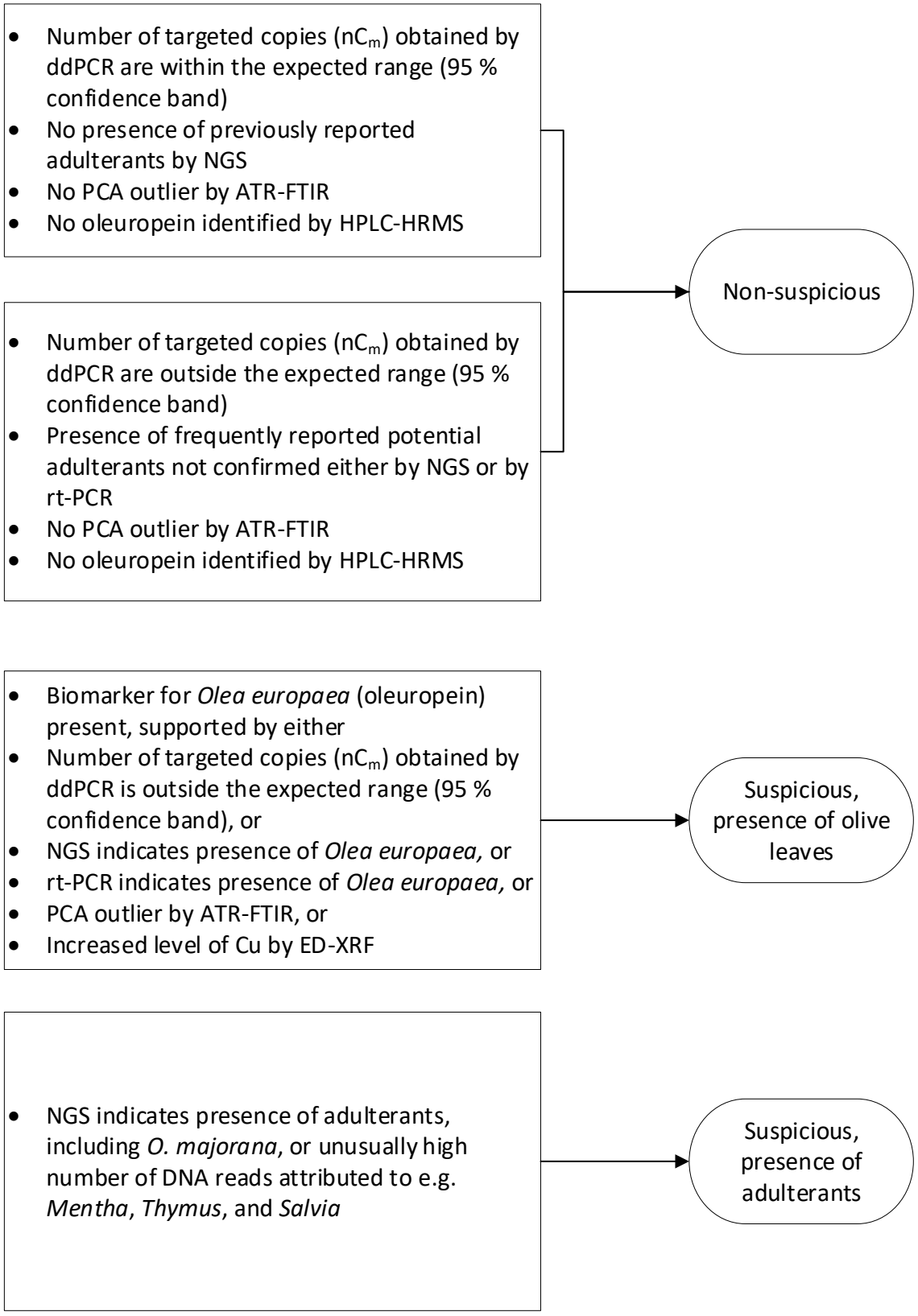
*) Black mustard (*Brassica nigra*), brown mustard (*B. juncea*), white mustard (*Sinapis alba*), radish (*Raphanus sativus*), black cumin *syn*. black caraway (*Bunium persicum*), caraway (*Carum carvi)*, coriander (*Coriandrum sativus*), fennel (*Foeniculum vulgare*), linseed (*Linum* spp.), pepper (*Piper nigrum*), squash/pumpkin (*Cucurbita pepo*), bindweed (*Convolvulus arvensis*), peanut (*Arachis hypogaea*) and starch containing bulking agents

Curcuma (*Curcuma longa*)

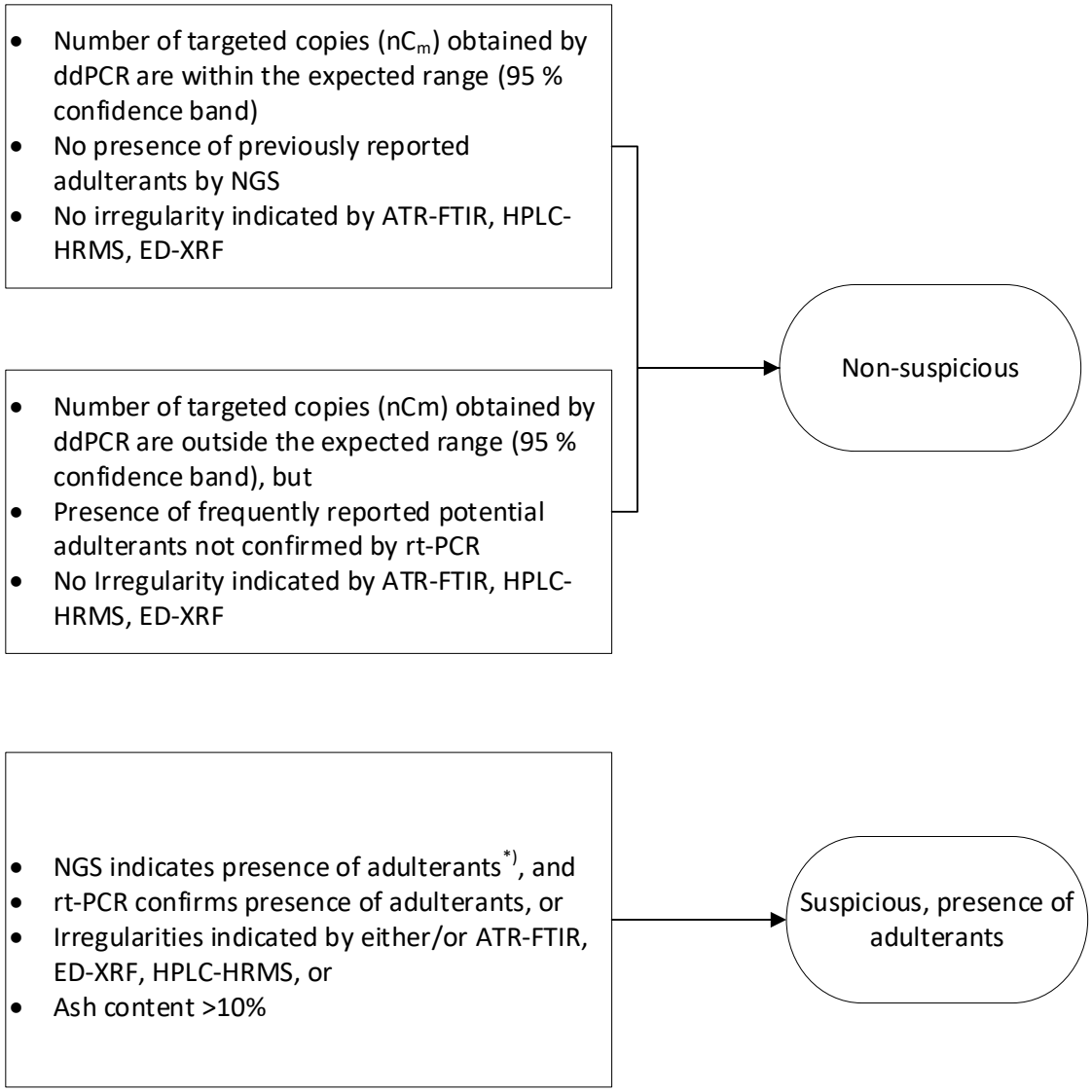


*) *Allium* spp., *Arachis hypogaea*, *Avena* spp., *Bunium persicum*, *Brassica* spp.,*Carum carvi*, *Capsicum* spp., *Cicer arietinum*, *Convolvulus arvensis*, *Coriandrum sativus*, *Cuminum cyminum*, *Glycine max*, *Gossypum hirsutum*, *Triticum* spp., *Piper nigrum*, *Solanum lycopersicum*, *Sinapis alba*, *Zea mays*

Oregano (*Origanum vulgare*)

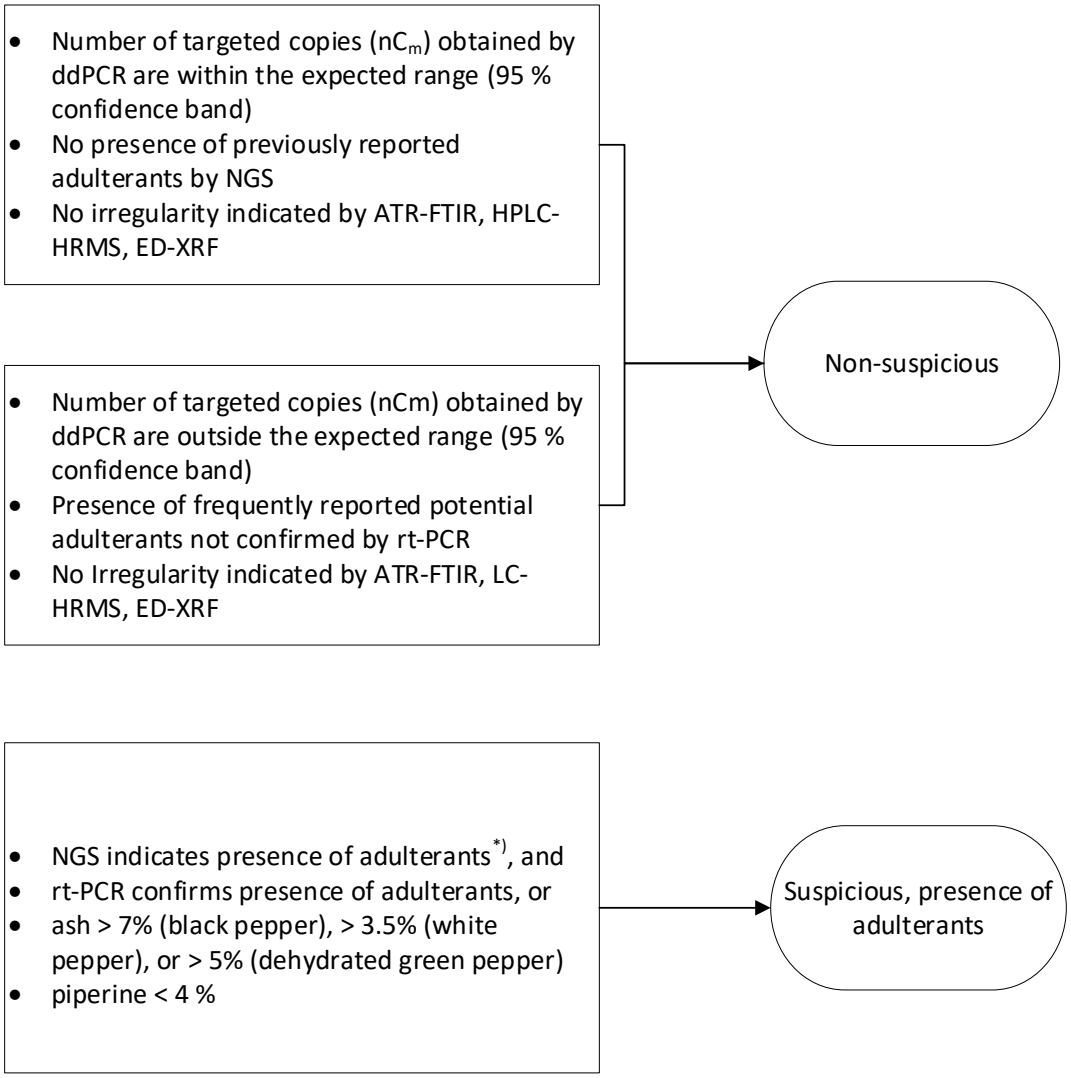


Paprika/chilli (*Capsicum annuum/C. frutescens*)



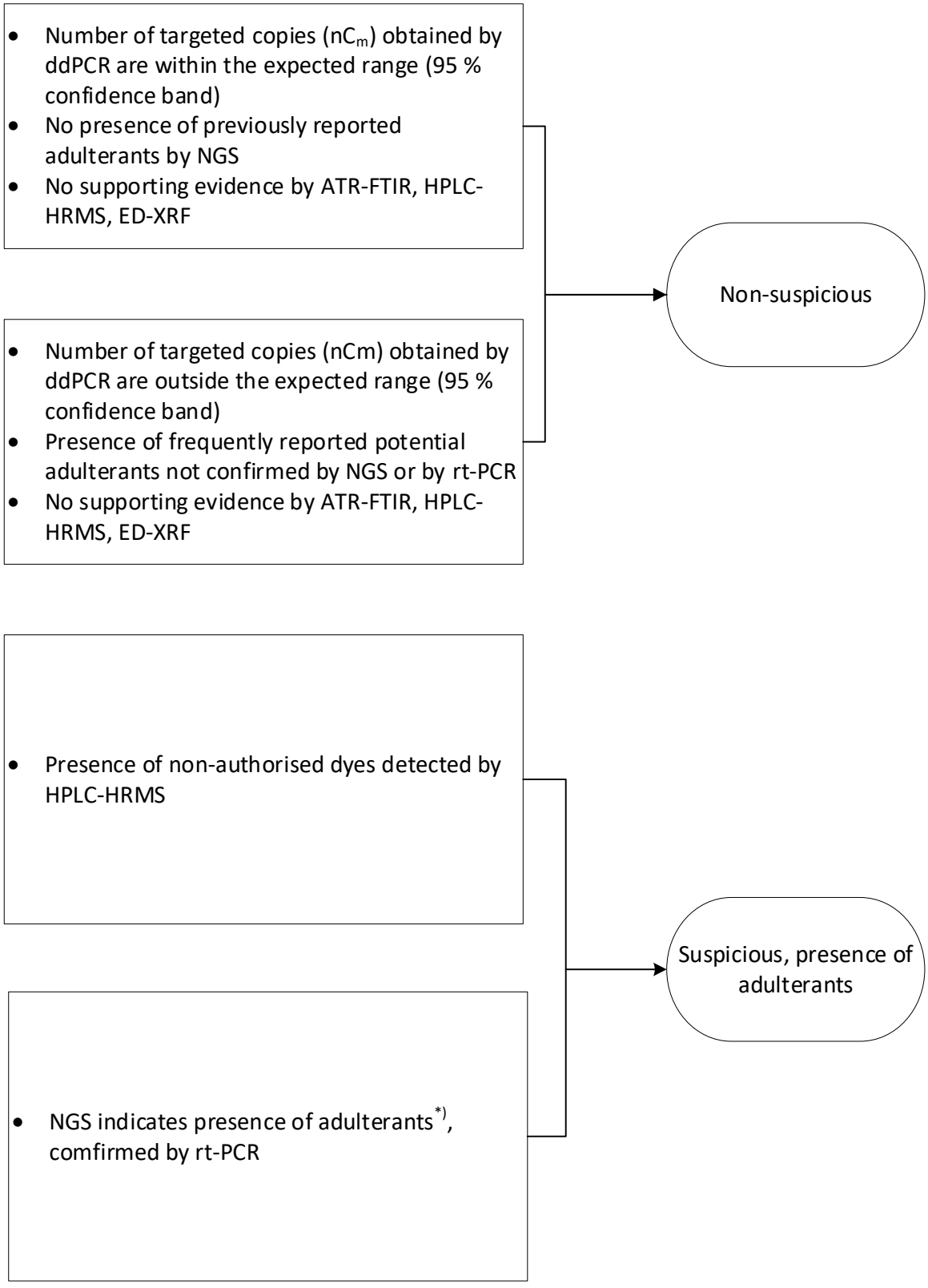
*) *Allium sativum*, *Chenopodium album*, *Coriandrum sativum*, *Cuminum cyminum*, *Daucus carota*, *Helianthus annuus*, *Prunus* spp., *Solanum lycopersicum*, *Zea mays*.

Pepper (*Piper nigrum*)



*) *Allium sativum*, *Brassica* spp., *Sinapis alba*, *Bunium persicum*, *Carum carvi*, *Capsicum* spp., *Carica papaya, Cicer arietinum, Coriandrum sativum*, *Cuminum cyminum, Fagopyrum esculentum, Foeniculum vulgare*, *Hordeum vulgare*, *Ipomoea* spp., *Origanum vulgare*, *Oryza sativa, Piper longum, Piper methysticum, Prunus* spp., *Trigonella foenum-graecum, Triticum aestivum, Zea mays,* and starch containing bulking agents.

Saffron (*Crocus sativus*)



*) Safflower (*Carthamus tinctorius*)

Annex 4. References

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