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Research Article

FOOD CHEMICAL CONTAMINANTS

A DNA Metabarcoding Workflow to Identify Species in Spices and Herbs

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Abstract

Background: Spices and herbs are food categories regularly cited as highly susceptible to be adulterated. To detect potential adulteration with undeclared species, DNA-based methods are considered the most suitable tools.
Objective: In this study, the performance of the ready-to-use Thermo Scientific™ NGS Food Authenticity Workflow (Thermo Fisher Scientific)—a commercial DNA metabarcoding approach—is described. The tool was further applied to analyze 272 commercial samples of spices and herbs.
Method: Pure samples of spices and herbs were analyzed with the Thermo Scientific NGS Food Authenticity Workflow to assess its specificity, and spikings down to 1% (w/w) allowed evaluation of its sensitivity. Commercial samples, 62 and 210, were collected in Asian and European markets, respectively.
Results: All tested species were correctly identified often down to the species level, while spikings at 1% (w/w) confirmed a limit of detection at this level, including in complex mixtures composed of five different spices and/or herbs. The analysis of 272 commercial samples showed that 78% were compliant with the declared content, whereas the rest were shown to contain undeclared species that were in a few cases allergenic or potentially toxic.
Conclusions: The Thermo Scientific NGS Food Authenticity Workflow was found to be suitable to identify food plant species in herbs and spices, not only when tested on pure samples, but also in mixtures down to 1% (w/w). The overall workflow is user-friendly and straightforward, which makes it simple to use and facilitates data interpretation.
Highlights: The Thermo Scientific NGS Food Authenticity Workflow was found to be suitable for species identification in herbs and spices, and it allowed the detection of undeclared species in commercial samples. Its ease of use facilitates its implementation in testing laboratories.

Among the cases of food fraud, species substitution and dilution with cheaper materials are the most reported, especially in the sector of spices and herbs (1, 2). Regulators and food industries must mitigate food fraud to ensure food quality and safety and to maintain consumers' trust; in addition to quality/traceability certifications and audits, suitable

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analytical techniques are therefore needed to verify product authenticity (3, 4).

To detect potential adulterants in spices and herbs, available analytical tools include (i) simple sensorial, visual, and microscopic methods; (ii) chromatographic methods, such as high-performance liquid chromatography and gas chromatography coupled with mass spectrometry; (iii) spectroscopic methods, such as Fourier transform infrared and Raman; and (iv) DNA-based methods (5, 6). For species identification, DNA-based approaches have gained more and more interest since DNA is present in all the plant tissues, is conserved through the evolution, and is relatively robust against industrial processes (7). Contrary to visual or microscopic methods, DNA-based methods can be applied on ground materials that are often produced and commercialized in the category of spices and herbs. In addition to species differentiation, DNA-based methodologies, as open and unbiased, can therefore further discriminate closely related species and different cultivars/varieties, which cannot be differentiated by any other microscopic, spectroscopic, or chromatographic approaches (6). As a limitation, DNA-based methods cannot detect nonbiological adulterants that do not contain DNA and are not applicable to detect different parts of the same plant species (such as leaves and roots) that share the same DNA (5).

With approximately 370 000 plant species registered by botanists (8), 30 000 edible and 150 being commercially cultivated (9), targeted approaches cannot be applied to detect and identify spices and herbs and their potential vegetal adulterants. Untargeted DNA-based analytical tools for species identification are based on DNA sequencing, using either DNA barcoding (10, 11) by Sanger sequencing or DNA metabarcoding by next generation sequencing (NGS). DNA barcoding can be applied on pure/single-species samples only, whereas DNA metabarcoding is applicable for mixtures and often on processed matrixes when amplifying fragments shorter than 400 base pairs (bp) (12). For this reason, DNA metabarcoding is becoming the method of choice for testing food authenticity (13, 14), and recent studies have shown its applicability for meat (15) and seafood (16) identification. The European Spice Association declared that DNA metabarcoding should be used as a secondary tool to detect potential adulteration with exogenous material containing DNA (17). At the same time, in recent years the species identification of botanical materials, including spices and herbs, has been described as increasingly reliable (18–20). Authorities are also moving toward DNA metabarcoding to identify plant species in herbs and spices; very recently, this approach was applied by the European Joint Research Center (JRC) on 1885 samples of herbs and spices from European countries (21). Beside this, very promising data have been progressively obtained for quantitative determination (22).

In this study, the performance of a ready-to-use commercial system, the Thermo Scientific™ NGS Food Authenticity Workflow (Thermo Fisher Scientific, Waltham, MA, USA), was evaluated as an untargeted platform to identify species from spices and herbs products. Combined with the SGS™ All Species ID Plant DNA Analyser Kit (SGS Molecular, Lisbon, Portugal), the workflow was applied to pure plant species and admixtures. Finally, 272 samples of spices and herbs were sourced from European and Asian markets and analyzed by the presented NGS workflow, to gain insight into adulteration in spices and herbs.

Experimental

Apparatus

(a) *DNA extraction.*—Some DNA extractions were performed on a Maxwell® RSC instrument (Promega Corporation, Madison, WI, USA).

(b) *NGS.*—NGS chips were loaded using an Ion Chef™ Food Protection instrument (Thermo Fisher Scientific, Waltham, MA, USA) and sequenced on an Ion GeneStudio™ S5 Food Protection sequencer (Thermo Fisher Scientific).

(c) *DNA concentration measurement.*—DNA concentration was measured on an Invitrogen™ Qubit™ fluorometer (Thermo Fisher Scientific).

Reagents and Materials

(a) *DNA extraction.*—DNA extractions were performed with the Maxwell RSC PureFood GMO and Authentication kit (Promega Corporation), or with the NucleoSpin Food kit (Macherey-Nagel GmbH, Duren, Germany), or according to Cottenet et al. (2020) (15).

(b) *DNA dilution.*—EB buffer (QIAgen, Hilden, Germany).

(c) *Plant NGS Library preparation.*—SGS™ All Species ID Plant DNA Analyser Kit (SGS Molecular).

(d) *NGS Library purification.*—Purification of NGS libraries was performed with Agencourt beads (Beckman Coulter, Nyon, Switzerland).

Samples

Pure leaves, grains/seeds, and tubers from different spices and herbs (Table 1) were obtained from local markets, as well as other plant species and meat and fish, commonly found in the food supply chain. When the identity of leaves or seeds was not certain, identification was confirmed by DNA barcoding (10) before using the sample. After a washing step under a water flow for a few seconds to remove potential dust/contaminants from other species, the materials (e.g., leaves or seeds) were ground, and 1 g was used for DNA extraction.

Mixtures were prepared by weighing separately the ground plant species to reach a total of 1 g. For example, 50 mg of parsley was added to 950 mg of oregano to obtain a 5% (w/w) parsley in oregano sample, and the overall mixture was further extracted.

Finally, 272 samples of spices and herbs commercialized as ground products were collected from local markets, 62 from Asian countries and 210 from European countries.

DNA Extraction

DNA from 1 g of sample was extracted with the Maxwell RSC PureFood GMO and Authentication kit using the Maxwell RSC instrument (Promega Corporation, Madison, WI, USA), or with the NucleoSpin Food Kit (Macherey-Nagel GmbH, Duren, Germany), or according to Cottenet et al. (2020) (15). DNA concentration was measured with the Invitrogen Qubit fluorometer (Thermo Fisher Scientific, Waltham, MA, USA), and DNA extracts were diluted at 10 ng/μL in EB buffer (QIAgen). All extraction protocols led to sufficient DNA amount and quality, as required for further DNA amplification and library preparation. Extracted DNA was stored at −20°C until further use.

Table 1. List of species tested and their corresponding Latin name reported by the Thermo Scientific NGS Food Authenticity Workflow[a]

Tested species	Reported by NGS	Tested species	Reported by NGS
Maize (G)	*Zea mays*	Oregano (L)	*Origanum vulgare/onites*
Soya (G)	*Glycine max*	Rosemary (L)	*Rosmarinus officinalis*
Wheat (G)	*Triticum aestivum*	Basil (L)	*Ocimum basilicum*
Rice (G)	*Oryza sativa*	Thyme (L)	*Thymus vulgaris*
Rye (G)	*Secale cereal*	Parsley (L)	*Petroselinum crispum*
Barley (G)	*Hordeum vulgare*	Chive (L)	*Allium schoenoprasum*
Oat (G)	*Avena sativa*	Marjoram (L)	*Origanum marjorana*
Millet (G)	*Panicum miliaceum*	Laurel (L)	*Laurus nobilis*
Sorghum (G)	*Sorghum bicolor*	Dill (L)	*Anethum graveolens*
Potato (T)	*Solanum tuberosum*	Fennel (L)	*Foeniculum vulgare*
Tomato (G)	*Solanum lycopersicum*	Fenugreek (G)	*Trigonella foenum-graecum*
Rapeseed (G)	*Brassica* spp.	Coriander (G)	*Coriandrum sativum*
Black mustard (G)	*Brassica* spp.	Anise (G)	*Pimpinella anisum*
White mustard (G)	*Sinapis alba*	Celery (L)	*Apium graveolens*
Black pepper (G)	*Piper nigrum*	Garlic (L)	*Allium sativum*
Turmeric (T)	*Curcuma longa*	Juniper berry (G)	*Juniperus communis*
Cumin (G)	*Cuminum cyminum*	Nutmeg (G)	*Myristica fragrans*
Caraway (G)	*Carum carvi*	Saffron (L)	*Crocus sativus*
Paprika/Chili (G)	*Capsicum spp. annuum*	Peanut (G)	*Arachis hypogea*
Ginger (T)	*Zingiber officinale*	Almond (G)	*Prunus spp.*
Carob (G)	*Ceratonia siliqua*	Argemone (G)	*Argemone spp.*
Tapioca (T)	*Manihot esculenta*	Hemlock (L)	*Conium maculatum*
Cardamom (G)	*Elettaria cardamomum*	Leek (L)	*Allium ampeloprasum*
Sesame (G)	*Sesamum indicum*	Sage (L)	*Salvia officinalis*
Opium poppy (G)	*Papaver somniferum*	Pea (G)	*Pisum sativum*
		Carrot (T)	*Daucus carota*
Beef (M)	No amplification/identification	Pink salmon (M)	No amplification/identification
Pork (M)	No amplification/identification	Cod (M)	No amplification/identification
Chicken (M)	No amplification/identification	Anchovy (M)	No amplification/identification
Sheep (M)	No amplification/identification	Whiting (M)	No amplification/identification
Goat (M)	No amplification/identification	Hake (M)	No amplification/identification

[a] Samples were collected as pure grains/seeds (G), pure tubers (T), pure leaves (L), or pure meat (M).

Library Preparation and NGS Run

The analysis of the extracted plant DNA was performed with the SGS All Species ID Plant DNA Analyser Kit, according to the manufacturer's instructions. Briefly, plant DNA was first amplified with two separate PCR tests, targeting different plant DNA targets (kept proprietary by Thermo Fisher Scientific and SGS Molecular) with variable sizes of approximately 200 and 300 bp depending on plant species. These targets include the recommended DNA regions to be used for plant species identification (23), namely *ITS2*, *trnL*, *matK*, and *rbcL*. Together with unique sample barcodes and kit barcodes, expected amplicons range from 250 to 350 bp when observed on gel electrophoresis. Amplicons were then pooled together and purified on magnetic Agencourt beads (Beckman Coulter, Nyon, Switzerland). The purified library, 50 pM, was loaded on an Ion 510 chip using the Ion Chef Food Protection instrument (Thermo Fisher Scientific), and then sequenced on an Ion GeneStudio S5 Food Protection sequencer (Thermo Fisher Scientific) set with a 400 templating size and a 600 flow rate.

The SGS All Species ID Plant DNA Analyser Kit included a positive control sample that allowed us to monitor the reliability of the overall analysis, to verify the correct assignment of the reads to each specific barcode used in the pooled NGS library, and to ensure that no cross-contamination with reads of different barcodes occur during the labratory workflow and data analysis.

Data Analysis

Once the run completed, the FASTQ file containing all the nucleotide sequences was uploaded in the SGS All Species ID software version 2.5.1 (SGS Molecular). This software automatically performs the reads de-multiplexing as well as the identification of plant species, by aligning the obtained sequences of each individual sample against a curated built-in database (version 5.0) that contains approximately 5000 plant species. A list of the species present in this database was provided by the supplier. Although it was not the purpose of this work, several FASTQ files were also analyzed with an in-house bioinformatic pipeline to verify the trueness of the SGS All Species ID software. This in-house pipeline included a read quality evaluation and a preprocessing of the FASTQ files using the fastp program (24), version 0.21.0, followed by a clustering of the reads by homology using the vsearch program (25), version 2.17.1. Finally, a representative sequence from each homology group was compared with the nucleotide NCBI database (26). Both the SGS All Species ID software and in-house pipeline led to similar results matching on two DNA regions (*ITS2* and intergenic *trnL* sequences),

thus demonstrating the reliability of the ready-to-use software (data not shown).

Results and Discussion

Pure Plant Species Identification

The specificity of the method was tested on plants, meat, and fish to verify that only plant material was amplified and identified. No amplification was observed on any of the meat and fish samples, whereas the two PCR reactions led to amplicons ranging from 250 to 350 bp when tested on plant samples (data not shown). After sequencing, species identification was performed by the SGS All Species ID software by analyzing the FASTQ files. Plant samples were in general identified down to the species level without any misidentification (Table 1). The vast majority of spices and culinary herbs belong to the *Apiaceae* and *Lamiaceae* families, respectively. Although these families are known to contain closely related species, the *ITS2*, *trnL*, *matK*, and *rbcL* target genes have been shown to be well adapted for reliable species identification (27, 28). However, within a genus, closely related species might have highly similar genetic sequences, and identification down to the species level may not be feasible; this is well known for Brassicaceae (e.g., cauliflower, cabbage, rapeseed, mustard species) (29). Randomly, the NGS workflow also reported species with very few reads associated (<0.5% of the sample reads), below the limit of detection of the kit communicated by the manufacturer; they were therefore not considered in the analysis.

Nevertheless, with a database limited to 5000 plant species, some species will not be identified by the software; as examples, avocado and star anise were successfully amplified but were not identified (data not shown). This limitation is currently being investigated by the supplier, who is (i) regularly extending the database, (ii) proposing to further analyze the FASTQ files by blasting them on the global NCBI nucleotide database. The first solution has the advantage of building a curated database but with limited species, whereas the second approach unfortunately relies on a noncurated global database with very broad species available and requiring more bioinformatics support for processing and interpreting the data.

Detection in Mixtures (Spiked Samples)

To evaluate the sensitivity of the method and its capability to detect several species in the same sample, different mixtures were prepared and analyzed (Table 2), covering different families and genuses of spices and herbs frequently consumed. All adulterants spiked at 5% (w/w) were successfully detected and identified in the 44 tested samples. Adulterants spiked at 1% (w/w) were correctly detected and identified in 93 samples among the 97 samples tested. With a true positive rate of 95.8%, higher than 95%, this indicates a limit of detection at 1% (w/w) (30). This limit is well aligned with the acceptable level of extraneous matter defined by the ESA Quality Minima document (31).

In addition, three complex mixtures containing an equal amount (200 mg) of five different species were prepared and analyzed (Table 3). All species were successfully detected and identified, indicating the capability of the tool to also identify several species in a complex mixture that could be very useful to analyze, for example, curry and/or seasoning preparations. Although the tool was here applied as a qualitative identification tool, the relative percentage of reads assigned to each species was found to be quite close to the expected 20% (w/w) (Table 3), except in the first mixture for juniper berries and nutmeg. Compared to leaves and grains, fruits/berries and hard seeds are known to contain less DNA and to be more complicated to extract due to lysis-resistant compounds such as starch, pectin, mucilage, and lignin (32, 33). The Thermo Scientific NGS Food Authenticity Workflow might not deliver precise quantitative data but could still provide an estimation of the relative species content. Additional work would be needed to evaluate its quantitative capabilities.

Table 2. Identification of adulterant species spiked at 1% or 5% (w/w) in pure herbs and spices, and in complex mixtures containing five species at 20% (w/w) each[a]

Matrix	Spiked adulterant species (% w/w)		Adulterant detected and identified (number of replicates)
Paprika	Turmeric	1	19/20
Turmeric	Ginger	1	3/3
Cumin	Caraway	1	5/5
Oregano	Thyme	1	3/3
Basil	Parsley	1	21/22
Turmeric	Coriander	1	20/20
Coriander seeds	White mustard	1	3/3
Nutmeg	Juniper	1	3/3
Coriander	Parsley	1	3/3
Parsley	Conium	1	3/3
Dill	Chive	1	3/3
White pepper	Garlic	1	3/3
Rosemary	Spinach	1	2/3
Rosemary	Laurel	1	2/3
Paprika	Turmeric	5	5/5
Turmeric	Ginger	5	5/5
Cumin	Caraway	5	7/7
Oregano	Thyme	5	5/5
Basil	Parsley	5	7/7
Black pepper	Juniper	5	5/5
Anise	Fennel	5	5/5
Wheat	Soya	5	5/5

[a]The main matrix species was always detected and identified by the method.

Table 3. Identification of adulterant species in complex mixtures containing 20% (w/w) of five different species

Mixture	Adulterant detected and identified (number of replicates)	Average of reads (relative % reads)
Cumin	5/5	13 687 ± 2288 (31%)
Caraway	5/5	14 653 ± 8476 (33%)
Coriander	5/5	13 239 ± 3516 (30%)
Juniper berry	5/5	1965 ± 825 (4%)
Nutmeg	5/5	1293 ± 118 (3%)
Basil	5/5	6358 ± 3798 (17%)
Parsley	5/5	9985 ± 4175 (27%)
Oregano	5/5	6448 ± 1802 (17%)
Marjoram	5/5	5330 ± 2290 (14%)
Rosemary	5/5	9079 ± 2482 (24%)
Laurel	5/5	17 265 ± 3694 (26%)
Chive	5/5	13 678 ± 1353 (21%)
Dill	5/5	12 313 ± 3992 (18%)
Fennel	5/5	10 916 ± 2019 (16%)
Thyme	5/5	12 458 ± 4081 (19%)

Industrial Treatments and NGS Limitations

The Thermo Scientific NGS Food Authenticity Workflow has been shown to reliably identify plant species in unprocessed samples, including in mixtures, but commercialized spices and herbs usually undergo industrial treatments to eliminate potential microbiological hazards. Although harsh processes are known to degrade DNA, fragments of 400 bp are still amplifiable when boiling or drying plant materials for several hours, when treating plant samples with acidic or alkaline solutions, or when irradiating up to 10 kGy (34). To preserve essential aromas and colors, weak or mild industrial processes are usually applied to herbs and spices (35). Since small DNA fragments (<400 bp) are amplified by the SGS All Species ID Plant DNA kit, the approach is still compatible with the industrial processes applied to the vast majority of herbs and spices and therefore well adapted for their identification. Pressurized steam sterilization that can be applied in extreme cases by some producers on specific spices and herbs may be an exception; it is known to be sometimes applied to black or white pepper to remove the external shell as well as to efficiently inactivate pathogens. Pressurized steam treatment is similar to autoclaving or canning processes, which are known to significantly degrade DNA when temperature exceeds 100°C with pressure equal to or higher than 1 bar. This will then challenge the use of DNA metabarcoding, but more globally the use of any kind of DNA-based methods for spices/herbs authentication. When the pressure applied is below 1 bar, though, fragments up to 300 bp have been shown to still be amplifiable (36). Pressurized steam sterilization is nevertheless not applied to all spices and herbs and not so frequently since it is known to lead to color modifications and perceptible odor differences (37).

Survey on Spices and Herbs Samples From Local Markets

To gain insight into the current situation of contamination or adulteration of commercial spices and herbs with undeclared species, the Thermo Scientific NGS Food Authenticity Workflow was used to analyze 272 products sold as powders and collected from local markets in Europe ($n = 210$) and Asia ($n = 62$), without any prior knowledge about industrial treatments that they have undergone. Among them, 22% were found to contain undeclared species, with the herb category being the most contaminated or adulterated. Oregano and basil were the most impacted products with more than half of the tested samples found to contain undeclared species, followed by laurel and thyme (Figure 1). Oregano has already been reported to be regularly adulterated due to its high cost (38, 39). In these samples, other aromatic herb species such as parsley and rosemary were detected (Table 4), which may be due to cross-contamination in the field during the harvest, in the factories, or at the raw material storage, processing, and/or packaging steps; cleaning volatile powders is a process known to be very complex when switching between different species. In addition, these samples were shown to be contaminated with well-known garden/field weeds such as burnet saxifrage (*Pimpinella major*) and bindweed species (*Fallopia convolvulus*, *Polygonum* spp., and/or *Convolvulus arvensis*), which are known to frequently contaminate crops and aromatic herbs and decrease the production yield (40). Dedicated actions should be taken when *C. arvensis* is detected due to its capacity to produce toxic tropane alkaloids (41). Finally, undeclared celery was also detected in two samples of thyme and parsley, which may lead to strong consumer health reaction due to its allergenic effect.

Compared to herbs where more than 50% of oregano and basil samples were found to contain undeclared species, spices were less problematic where a maximum of 26% of the tested samples contained foreign species (Figure 1). Wheat and rice were often detected in white pepper, whereas coriander and fenugreek were detected in several black pepper powders (Table 4). Adulteration of white pepper powder with cheaper corn, wheat, or rice flour has already been mentioned as a topic of concern (42). In addition, one black pepper powder was shown to contain celery, which was correctly declared on the label as potentially present. Although foreign species were detected in only three ginger powder samples out the 15 tested, two of them presented a very limited amount of NGS reads (<5% of reads) attributed to ginger; in these two suspicious ginger powders, most of the reads were surprisingly identified as cowpea, cumin, and coriander, indicating a potential substitution of the ginger material. Cowpea powder has indeed a yellowish color that may mimic ginger powder and could serve as a clear adulterant (39). In addition, peanut (*Arachis hypogea*) was also reported in one of these two ginger samples, which can lead to critical allergenic reaction for sensitive consumers.

Paprika/chili samples were the most compliant samples and could be considered to be pure, except one sample from Asia that had several reads matching wheat (*Triticum aestivum*).

Overall, this survey revealed that 22% of the tested samples contained undeclared species, mostly edible plants. Interestingly our findings were well aligned with the recent survey from the European JRC (21), though we have obviously tested different physical samples. Not only was the proportion of suspicious samples very close, but the list of foreign species detected was also very similar, which demonstrates the validity of our findings. In addition to safe plants, the Thermo Scientific NGS Food Authenticity Workflow detected allergenic species (celery and peanut) as well as an alkaloid-producing contaminant (*C. arvensis*) in several herb samples, which can have a critical impact on consumers' safety. The presence of these species should be confirmed by dedicated analytical tools, either using allergen or chemical-specific tests, respectively. Although limited samples from Asia were collected, 29% of them contained undeclared material, whereas 20% of the European ones were shown to contain foreign species. More samples from Asia should be tested to have a more representative insight of the purity of the samples coming from this region, and samples from the American continent could be included in a future survey to have a global overview.

Even though 22% of the tested samples were shown to contain foreign material, the majority of the samples were considered pure and compliant with their declared content.

Conclusions

The Thermo Scientific NGS Food Authenticity Workflow was found to be suitable to identify food plant species in herbs and spices, not only when tested on pure samples, but also in mixtures down to 1% (w/w). The tool will therefore be able to assess the purity of a plant sample ≤99% (w/w) but will not be able to detect contaminants present at a level below 1% (w/w). At first sight, the evaluation of quantitative data indicated that precise quantification might be biased; nevertheless, the NGS Food Authenticity Workflow might still provide an estimation of the relative species content. While additional work would be needed to determine its quantitative capabilities, other methods should be used to precisely determine the degree of

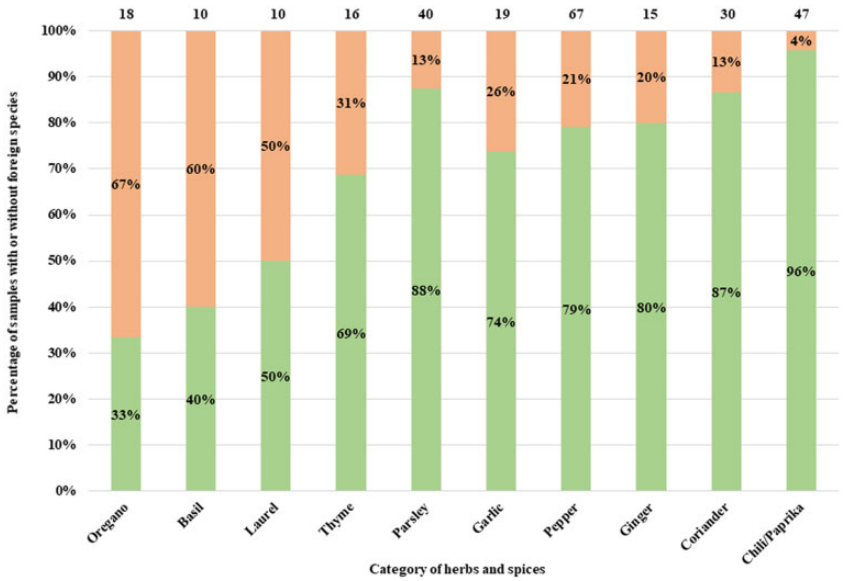

Figure 1. Percentage of samples per herb and spice category that did not contain any foreign species (green bars) versus the ones where undeclared species were detected (orange bars). The number of tested samples is indicated at the top of each bar.

Table 4. NGS findings in samples of herbs and spices collected in Europe and Asia[a]

Declared species	Samples collected (Europe/Asia)	With foreign species (Europe/Asia)	List of foreign species reported
Oregano (*Origanum onites/vulgare*)	18 (12/6)	12 (7/5)	**Bindweed (*Convolvulus arvensis*)**, Black-bindweed (*Fallopia convolvulus*), Baconweed (*Chenopodium album*), Lettuce (*Lactuca sativa*), Alfalfa (*Medicago sativa*), *Amaranthus* spp., Parsley (*Petroselinum crispum*), Thyme (*Thymus vulgare*), Rosemary (*Rosmarinus officinalis*), Salvia (*Salvia officinalis*)
Basil (*Ocimum basilicum*)	10 (7/3)	6 (4/2)	**Bindweed (*Convolvulus arvensis*)**, Parsley (*Petroselinum crispum*), Wireweed (*Sida* spp.),
Laurel (*Laurus nobilis*)	10 (10/0)	5 (5/0)	Burnet-saxifrage (*Pimpinella axifrage*), *Capsicum* spp., Rosemary (*Rosmarinus officinalis*), Caraway (*Carum carvi*)
Thyme (*Thymus vulgare*)	16 (10/6)	5 (2/3)	*Origanum* spp., Rosemary (*Rosmarinus officinalis*), Parsley (*Petroselinum crispum*), Bindweed (*Convolvulus arvensis*), Turmeric (*Curcuma longa*), **Celery (*Apium graveolens*)**
Parsley (*Petroselinum crispum*)	40 (33/7)	5 (3/2)	Black-bindweed (*Fallopia convolvulus*), *Solanum* spp., **Celery (*Apium graveolens*)**
Garlic (*Allium sativum*)	19 (17/2)	5 (5/0)	Laurel (*Laurus nobilis*), Onion (*Allium cepa*)
Ginger (*Zingiber officinale*)	15 (8/7)	3 (1/2)	Cowpea (*Vigna unguiculata*), Cumin (*Cuminum cyminum*), Coriander (*Coriandrum sativum*), Garlic (*Allium sativum*), **Peanut (*Arachis hypogea*)**
White/Black pepper (*Piper nigrum*)	67 (55/12)	14 (12/2)	Wheat (*Triticum aestivum*), Rice (*Oriza sativa*), Cumin (*Cuminum cyminum*), Coriander (*Coriandrum sativum*), *Brassica* spp., Fenugreek (*Trigonella foenum-graecum*), **Celery (*Apium graveolens*)**
Coriander (*Coriandrum sativum*)	30 (21/9)	4 (3/1)	Fenugreek (*Trigonella foenum-graecum*), *Origanum* spp., Black-bindweed (*Fallopia convolvulus*), Knotweed (*Polygonum* spp.), Parsley (*Petroselinum crispum*), *Brassica* spp.
Chili/Paprika (*Capsicum* spp.)	47 (37/10)	6 (4/2)	Wheat (*Triticum aestivum*), Onion (*Allium cepa*), Garlic (*Allium sativum*)

[a] Foreign species detected and identified with the Thermo Scientific NGS Food Authenticity Workflow in these commercial samples are reported per declared species as a whole, not per sample analyzed. Toxic or allergenic species are indicated in bold.

adulteration. The overall workflow is user-friendly and straightforward, which makes it simple to use and facilitates data interpretation. However, one can criticize the lack of access to proprietary information that is kept confidential, such as the primers used and the algorithms that are applied in the SGS All Species ID software. The major edible plant species are included in the curated database and were successfully identified in our study; nevertheless, its current limitation to approximately 5000 species could prevent the tool from identifying more exotic plant species and potentially toxic ones.

To gain insight into the current situation of commercial spices and herbs, the Thermo Scientific NGS Food Authenticity Workflow was applied to analyze 272 products. Among the 22% of the samples found to contain foreign species, two ginger samples were suspected to be substituted with other materials, four products were found to contain allergenic plant materials, and the others contained other edible species, most probably due to cross-contaminations along the supply chain. Interestingly, these findings were well aligned with the last survey from the European JRC, though different physical samples were obviously tested. Further work on herbs and spices should be conducted to clarify and define legal thresholds in order to differentiate between supply chain cross-contamination and intentional adulteration for economical purposes. Also, with the growing interest in species identification by DNA metabarcoding, the impact of industrial treatments on the DNA of herbs and spices should be more thoroughly evaluated to understand potential analytical biases and limitations. Here, even though samples came from various commercial markets without knowing in advance their industrial treatments, tangible results were obtained with the Thermo Scientific NGS Food Authenticity Workflow, aligned with previous studies.

To conclude, the Thermo Scientific NGS Food Authenticity Workflow applied to the identification of spices and herbs was able to reliably identify the different varieties of species down to 1% (w/w), and it was well adapted to analyze real-life samples and to detect cases of contamination and/or adulteration with undeclared species. This tool can be easily implemented in food testing laboratories to analyze the authenticity of plant materials, and it can further be used for meat and fish species identification.

CRediT Author Statement

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Conflict of Interest

All authors declare no conflict of interest.

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