PROJECT FINAL REPORT

Grant Agreement number: 312631

Project acronym: SPICED

Project title: Securing the spices and herbs commodity chains in Europe against deliberate, accidental or natural biological and chemical contamination

Funding Scheme: Collaborative Project (CP) – Small or medium-scale focused research project (STREP)

Period covered: **from** M1 (01 July 2013) **to** M36 (30 June 2016)

Name of the project coordinator, Title and Organisation:

Dr. Juliane Bräunig, Federal Institute for Risk Assessment (BfR)

Tel: +49 30 18412 2142

Fax: +49 30 18412 2951

E-mail: juliane.braeunig@bfr.bund.de

Project website address: www.spiced.eu

Final publishable summary report

Executive summary

The overall objective of the European Union (EU) funded project SPICED has aimed at securing the spice and herb food chains from primary production to consumer-ready food against major natural, accidental or deliberate contaminations. Within SPICED, focus was on the low-moisture food ingredients dried herbs and spices.

The EU market is one of the largest markets for spices and herbs in the world. Mostly, these commodities are imported as dried raw materials from producing regions outside of the EU. Despite the low water activity, which inhibits microbiological growth, spices and dried herbs are natural products that can be contaminated with several microorganisms among them pathogenic and toxigenic species. Moreover, chemical contaminations may occur. These mainly result from natural or unintentional inclusions, yet may also transpire from economic benefits.

Contaminations with microbiological and chemical agents can take place at numerous vulnerable points within production and supply chains and can pose a serious risk for the consumer. As quantitatively minor food ingredients, spices and herbs hold a major potential to contaminate a wide range of products due to the wide-spread use and large-scale distribution. However, the identification of contaminated spices and herbs as a cause of a food-borne infection or intoxication would be difficult, because consumers and experts often focus on major food ingredients. Moreover, many detection methods are less suitable for the heterogeneous spice/herb matrices.

The research objectives of the SPICED project have focused on i) characterising the heterogeneous matrices of spices and herbs based on their respective production and supply chains concerning relevant biological and chemical hazards that can lead to major natural, accidental or deliberate contaminations in the food chain. Furthermore, focus was set on ii) improving the knowledge on biological hazard properties as well as on-site and high throughput diagnostic methods for appropriate agent detection, and iii) reducing (industrial) chemical adulterations as well as ensuring the authenticity of spices and herbs by evaluation and improvement of non-targeted fingerprinting methods. Additionally, the project has focused on iv) improving alerting, reporting, and decontamination systems as well as techniques to ensure prevention and response of a high quality.

To achieve the project objectives, SPICED has integrated relevant stakeholders from public and private bodies including the civilian and the military sectors in a unique consortium. The consortium is composed of eleven project partners and four integrated stakeholders, covering experienced institutions from industry, academia, and food authorities from seven EU member states.

Summary description of project context and objectives

Minor food ingredients, such as spices and herbs, are often not a focus during a food-borne incident. Moreover, low moisture food like dried herbs and spices can be underestimated regarding biological hazards since microorganisms are not able to proliferate at low water activity levels. However, some microorganisms, including pathogenic and toxigenic ones, can survive even in dried herbs and spices. When added to food with higher moisture content without further processing to inhibit or inactivate microorganisms, microbial proliferation is possible upon improper food storage.

Besides biological hazards, spices and herbs can contain chemical agents of concern. These cover natural and unintentional contaminants/residues, such as mycotoxins, heavy metals or pesticide residues. Moreover, intentional contamination can occur. Most prominent examples are potentially carcinogenic Sudan dyes added illegally to low-quality spices (particularly to paprika/chilli powder and curcuma) to improve the colour. Spices and herbs are valuable products and authentication is of particular importance for product quality as well as safety to protect the consumer and to avoid economic losses.

To prevent contaminations, knowledge on the potential entry points for contaminations within the food chain is necessary. Mostly, spices but also herbs are imported as dried raw materials from producing regions outside of the EU. Their chains include several critical points in processing and supply. However, the detection of potential health hazards in spices and herbs can also be a challenge. On the one hand, contaminations often occur heterogeneously within a batch making proper sampling methodologies crucial. On the other hand, the detection itself can be difficult. Spices and herbs are characterised by a high content of phenolic compounds. Such compounds can interfere with detection methods and can also exhibit antimicrobial activity. Thus, enrichment of microorganisms prior detection is often not possible in spice/herb matrices or requires an improved methodology. Moreover, the detection method, such as PCR, can be more difficult or inhibited.

Proper sampling, sample preparation and detection methods are crucial for product testing and monitoring. In case of microbiological contaminations, microbial reduction treatments can be applied if levels exceed the limits. If products that have been placed on the market do not comply with legal obligations, reporting and alerting systems like the European Rapid Alert System for Food and Feed (RASFF) are important tools to prevent further spread of potentially harmful foodstuffs. Proper reporting is necessary to enable tracing of products, which is of crucial importance in case of a food-borne incident/outbreak.

The SPICED project aimed at securing the spices and herbs commodity chains in Europe against deliberate, accidental or natural biological and chemical contamination. The overall objectives of SPICED have been:

1) To characterise the heterogeneous matrices of spices and herbs and their respective production and supply chains in context with relevant biological and chemical hazards that can lead to major natural, accidental or intentional contaminations in the food supply chain

Spices and herbs are of specific relevance, as they are natural products that can be contaminated with various microorganisms and chemical agents, among them pathogenic species and toxic substances. Furthermore, spices and herbs are added to almost every processed food, including ready-to-eat products. Therefore, condiments can be a risk factor since consumers can be directly exposed to potential health hazards, when contaminated spices and herbs are added to the food and no further inactivation step takes place.

Europe has one of the largest markets for spices and herbs in the world; however, these commodities are mainly produced outside of the EU. Most of the raw materials are imported in a dried and crude condition, before they are further processed at European spices and herbs processors. Hence, the European market is depending on the product quality of non-European producers. As high quality finished products cannot be made from poor quality raw materials, it is of main importance that the first step in the process is to check each batch of incoming raw material to ensure that the quality is congruent with public and private standards.

The first main project objective was addressed by Workpackage (WP) 2. The aim of WP 2 was to systematically collect and evaluate information on spice/herb producing operations as well as on hazards with the potential for natural, accidental and/or deliberate spice/herb contamination. In addition to collecting information, identifying vulnerable points in the spice/herb production chains and assessing the survival capacities of agents have been performed. Furthermore, WP 2 aimed at evaluating sampling strategies to detect microbiological or chemical hazards. Additionally, the most relevant spice matrices and agents for deliberate and natural contaminations have been identified.

The analysis of the current status of supply and processing chains enables the evaluation of the possible spread of contaminants. In combination with the standard inclusion rates of spices and herbs in products and dose-response estimations, risks and potential interventions have been determined.

2) To improve the knowledge on biological hazard properties as well as on-site and high throughput diagnostic methods for appropriate detection

The second main objective was addressed in WP 3. The major challenges of WP 3 were the characterisation of the biological properties and the development of reliable and standardised detection methods for biological hazards in spices and dried herbs. To ensure that the newly developed techniques will be based on the most current diagnostic methods, a method database was established.

In the practical part of WP 3, on-site screening and detection methods have been investigated to ensure optimised testing of samples in surveillance and suspicious consignments. Additionally, high throughput detection methods have been developed and established for the generation of data about the prevalence and tenacity of respective biological agents. Of additional interest were approaches with large prospects for the discrimination between living and non-living bacteria, and for rapid quantification and broad-range detection of biological hazards, all which have been investigated for selected agent–matrix combinations. For standardisation and harmonisation of diagnostics, a ring trial has been carried out.

3) To reduce (industrial) chemical adulterations and to ensure authenticity of spices and herbs by evaluation and improvement of non-targeted fingerprinting methods

Traditional strategies to guarantee food quality and safety and to detect adulterations are typically based on wet chemistry in order to determine certain marker compounds. These approaches suffer from a number of disadvantages, namely, the ever-increasing number of analytes, which must be included in any test procedures and the limited knowledge of the range of each constituent in normal, unobtrusive materials. A further limitation of such targeted approaches is their insufficient capacity to detect unforeseen agents and contaminants. As such, there is a continuing demand to investigate new ways of combining existing test methods and emerging technologies into a comprehensive analytical strategy for early quality and safety assurance in the food chain. An upcoming, innovative alternative approach to investigate any kind of distinctive features is based on non-targeted analytical techniques (fingerprinting techniques) in combination with chemometric modelling.

Establishing such an approach was the focus of WP 4, which aimed at developing a rapid and cost-efficient set of methodologies for the detection of natural, accidental and deliberate contamination of spices and herbs with (unforeseen) chemical agents. For this purpose, several spectrometric and spectroscopic fingerprinting techniques were applied to a representative set of authentic as well as adulterated spice and herb samples. The techniques included proton-transfer-reaction mass spectrometry (PTR-MS), high resolution mass spectrometry hyphenated to high performance liquid chromatography (HPLC-HRMS), inductively coupled plasma mass spectrometry (ICP-MS), nuclear magnetic resonance spectroscopy (NMR) and Fourier transform infrared spectroscopy (FT-IR). The fingerprinting data have been used to develop and evaluate various chemometric (one-class) classification techniques that allow distinguishing authentic (non-adulterated) samples from adulterated/contaminated samples. In addition, the application of HPLC-MS/MS to determine the identity and composition of the contaminants has been evaluated.

The research conducted within WP 4 provides a clear indication for the potential use of fingerprinting techniques in combination with chemometric modelling for food defence purposes and early detection of natural, accidental and deliberate contamination.

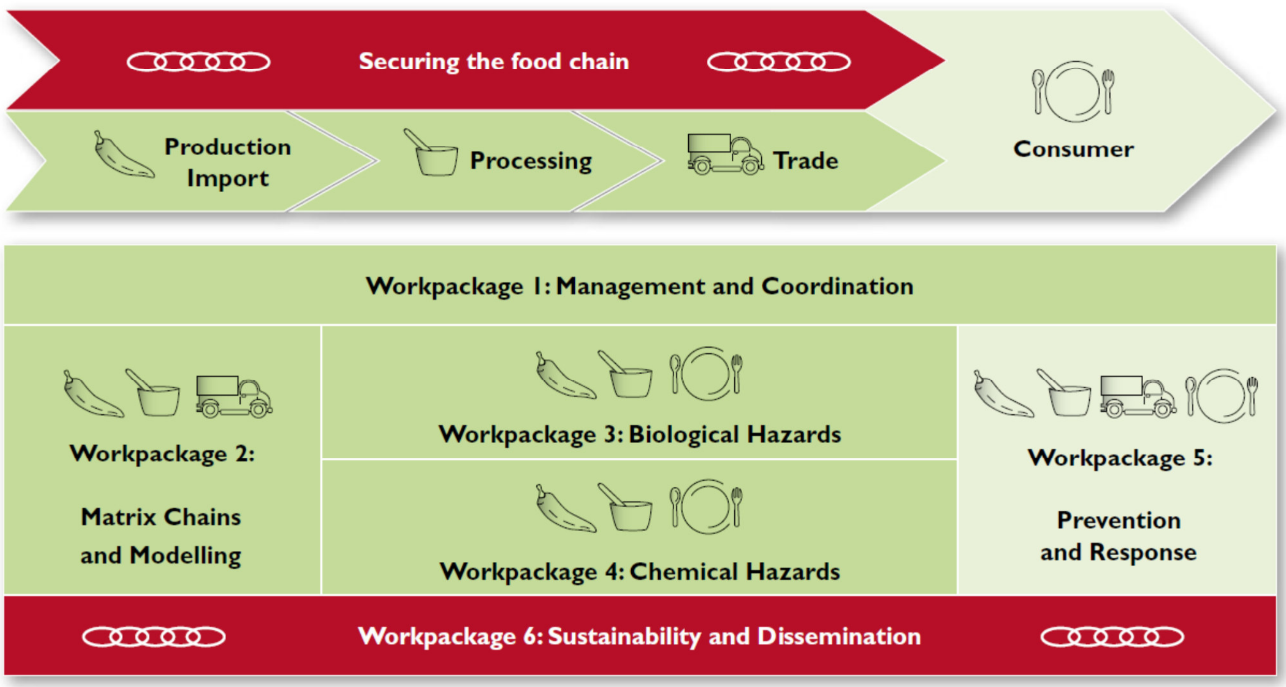
4) To improve alerting, reporting and decontamination systems as well as techniques to ensure prevention and response on high quality level

In general, food-borne incidents should be recognised as soon as possible to prevent a further spread. This needs an effective monitoring programme. In the instance of a food-borne outbreak or the exceedance of regulatory limits, the identification and tracing of commodities is most relevant. Even more, these should be accomplished promptly.

This last project objective falls within WP 5. This workpackage aimed at investigating recording and reporting mechanisms for commodity flows and food safety hazard data on the basis of legal regulations and private quality management systems to establish best practice examples. Based on these examples, recommendations for a standard electronic record system have been developed to analyse these data in a bidirectional manner (forward and backward tracing). Moreover, data from the European Rapid Alert System for Food and Feed (RASFF) and governmental monitoring systems have been evaluated for their suitability as alerting and early warning systems within the EU. Further, a guideline for the differentiation between natural and intentional incidents has been developed. In addition, WP 5 aimed at investigating and evaluating currently available decontamination methods for spices and herbs as well as for production facilities. Information on microbial reduction treatments have been collected and allocated in a database.

Description of the main results/foregrounds

The work within SPICED was structured in six workpackages (see scheme below). All necessary management activities within the SPICED project (scientific management and assessment of progress and results, financial and administrative management, intra-consortium communication, and management of gender issues) have been conducted on a professional basis with the European Commission, the coordinator, and the project partners within Workpackage 1 (WP 1). Intensive research work on the project's tasks has been carried out within four workpackages (WP 2 to WP 5). The dissemination activities performed within Workpackage 6 (WP 6) addressed the communication of the project objectives and its outcome to relevant stakeholders from public and private bodies including the civilian and military sectors.



The structure of the project spanning from primary production and import to the consumer within all workpackages.

<u>Matrices and agents in the focus of SPICED</u>

At the beginning of the project, the most relevant target matrices and the biological agents and chemical agents were defined by the consortium. As a result of the following facts, the matrices investigated in the SPICED project were pre-selected to ensure comparable results within the project.

- At least 400 spices and herbs are commercially available worldwide (heterogeneous matrix).
- The variability of production, processing, and distribution chains are unlimited from various places of raw material origin to the differing sales and distribution possibilities of the condiments (heterogeneous supply chain).
- A wide range of possibilities of manufacturing methods can be used to produce huge numbers of product types (heterogeneous production/processing chain).

For SPICED issues related to biological hazards, the following spices and dried herbs were identified to be the most relevant:

- paprika/chilli,
- black pepper,
- nutmeg,
- cinnamon/cassia,
- allspice/pimento,
- parsley,
- oregano leaves,
- basil leaves (sweet).

For SPICED issues related to chemical hazards, the following spices and dried herbs were considered to be most relevant:

- paprika/chilli,
- black pepper,
- nutmeg,
- saffron,
- vanilla,
- oregano leaves,
- basil leaves (sweet),
- thyme leaves.

The selection was based on various criteria such as i) the amount produced and consumed in Europe, ii) the potential to harbour natural, accidental or deliberate contaminations, and iii) the frequency of notifications related to such contaminations.

Additionally, several biological agents were chosen depending on the frequency of natural occurrence, the possible impact on human health, and the relevance for food terrorism. The biological agents in focus of SPICED particularly covered:

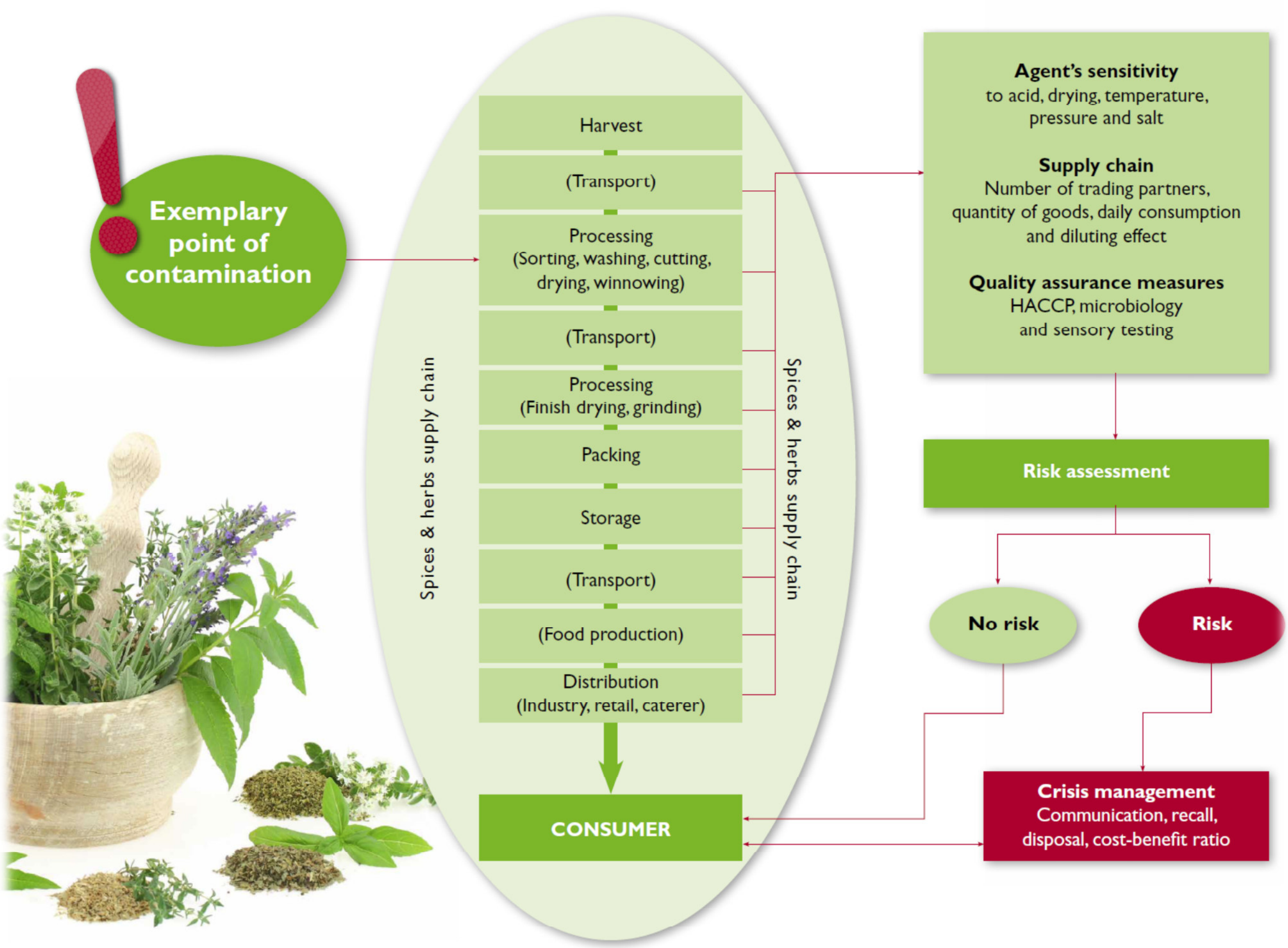
- *Salmonella* spp.,
- *Bacillus* spp.,
- *Listeria monocytogenes,*
- *Escherichia coli,*
- *Brucella microti,*
- *Clostridium perfringens,*
- *Staphylococcus aureus,*
- *Staphylococcus aureus* enterotoxin B (SEB),
- ricin.

The occurrence and the relevance for human health of chemical hazards were also evaluated. However, the focus for chemical hazards lies in the area of the non-targeted detection and ensuring authentication of the product. Nevertheless, to evaluate the possibilities and limitations of detecting adulterations in spices and herbs by fingerprinting techniques it was agreed to investigate the following scenarios:

- adulteration of nutmeg with nutmeg spent,
- adulteration of paprika/chilli with the illegal colourants Sudan I and Sudan IV,
- adulteration of dried oregano with olive leaves.

Within all the research objectives, systematic data acquisition and evaluation was performed for the spices and dried herbs, for the biological and chemical hazards, and for the chemical adulteration scenarios that had been selected.

This strategy also has been applied to the relevant topics in **WP 2 "Matrix Chains and Modelling"** that address the characterisation of biological and chemical hazards, predictive microbiology, evaluation of sampling strategies for the detection of hazards, and the analyses of spice chains including their vulnerable points.



Categorising biological and chemical hazards within spices and herbs

In order to develop a ranking system for the prioritisation of the biological and chemical agents with the potential for natural, accidental and/or deliberate contamination of spices and herbs, an extensive literature research was conducted.

For relevant biological hazards, a specific focus on the categorisation of relevant spices and herbs were considered according to the following criteria:

- various risk ranking methods,
- biological hazards of concern (natural, accidental and intentional contamination),
- dried culinary spices and herbs of concern,
- prevalence of contamination of the pathogen in the specified food,
- frequency and severity of disease,
- size and scope of production and consumption,
- diversity and complexity of the production chain and industry,
- potential for amplification of food-borne pathogens through the food chain.

However, it has to be recognised that microbial risk assessment is still a developing science and depends strongly on the availability of data as well as the question to be answered. In addition, emerging and re-emerging food-borne hazards that pose (new) risks for consumers change over time and are an increasing challenge to those working in the area of food safety. Moreover, changes in production and processing methods throughout the food chain and the evolution of consumption patterns as well as demands are contributing factors. Also, the expansion of international trade in food has in general increased the probability for microbiological hazards. Considering these factors, it has to be emphasised that the ranking lists developed for biological hazards are representing the *status quo* of data and information available and can change over time.

Furthermore, a list of chemical hazards of concern in spices and herbs considering suggestions from previous SPICED project results (WP 2, WP 4, and WP 5) and from industry partners was formulated. In addition, the investigated exposure to some chemical agents (mycotoxins, pesticides, dyes, etc.) in national monitoring, alerting and surveillance systems was investigated. During the literature review, some natural (plant) toxins were included in the list as these are not included in monitoring programmes. Literature and data were collected from scientific publications, alerting and (national) monitoring data, and other relevant EU reports and databases to determine the severity and exposure of the various chemical hazards. Given the available data and project resources, the risk ranking toolbox, developed within a project of the European Food Safety Authority (EFSA), was utilised to select an appropriate ranking scheme. A risk matrix approach was applied to chemical hazards in selected spices and herbs.

Currently, two scientific papers on prioritising biological as well as chemical hazards in the spice and herb chains are under preparation.

Development of a database on agents, matrices and processes

To increase the knowledge on the behaviour of microbiological contaminations in spices and dried herbs, a database has been developed, which covers relevant parameters of agents, matrices, and processes related to spice/herb chains. The database includes data on the microbiological agents in the focus of SPICED. Particularly, it provides information on the effect of different processes on microbiological contaminants. The processes mainly cover storage and certain decontamination treatments of dried herbs and spices. Moreover, the effects of several other processing treatments are included in the database, such as washing, heating for drying purposes, and special packaging. If available, the impact of these processes on the matrix characteristics was included. The database also covers data on the impact of different water activity levels on the microbial growth in spices. The considered studies were mostly performed with spiked condiments. However, in addition, some data on natural microbial contaminations are included. Next to literature data, the database integrates data obtained within SPICED and other unpublished data of the project partners. The database is provided as an Excel file. It consists of different spreadsheets, of which one contains the main data. Supplementary information on methods and information to support data entry are provided in additional spreadsheets. Moreover, the current database includes information on spice/herb consumption and standard inclusion rates of condiments in selected foods.

<u>*Predictive microbiology – agent survival during processing, transport, and storage*</u>

To evaluate pathogen survival along the spices and herbs chain and the effect of potential control options, so ultimately achieve public health protection, the concept of Food Safety Objective (FSO) with its factors, initial numbers (H_0), minus the sum of Log Reduction (ΣR) plus the sum of the Log Increase (ΣI), and its link to Appropriate Level of Protection (ALOP) was used.

The work included literature review, collecting data on spices and herbs regarding dose response of pathogens, prevalence, levels and distribution of microorganisms in spices and dry herbs, and also, inactivation/decontamination and survival of pathogens in the food chain. In addition, laboratory experiments were conducted to investigate microbial distributions, pathogen survival during decontamination (irradiation) and survival capacity in storage conditions, and the corresponding variability of the factors. Chosen pathogens for evaluation and for the experiments were *Salmonella* Infantis, *Listeria monocytogenes* and *Bacillus cereus*. The main matrices under investigation were paprika powder and powder of dry oregano. Other results of laboratory work include detection, spiking and sample preparation methods.

Literature analysis on sampling statistics and initial distribution of pathogens in dry food matrices was conducted. From the analysis, *Salmonella* spp. and *Bacillus cereus* were found of main relevance for spices and herbs. The prevalence of *Salmonella* spp. was at 3% for all spices investigated. Only few studies were found to contain also data on the actual levels of *Salmonella* spp. in spices. In those cases, levels of *Salmonella* spp. were found around -3 log Most Probable Numbers (MPN) per gram. Levels found at relevant outbreaks were at 0.5 log MPN/g. By using the MPN technique, the presence of *Salmonella* spp. (which was absent) was investigated in an industrial batch of oregano. The distribution of Enterobacteriaceae, sporeformers, *Bacillus cereus* and total viable count (TVC) was also investigated in the same batch. In general, mainly for the latter, high numbers were detected (e.g. total viable count aerobic: 5.3 ±0.2 log, Enterobacteriaceae: 3.9 ±0.4 log). From that analysis, lognormal distribution can be considered as representative distribution for all categories of the microorganisms tested. In this manner, both the mean level and the variation were characterised.

Further, meta-analysis on inactivation was conducted (more information on microbial reduction treatments is provided under “WP 5”). Results on irradiation indicate that despite the large variability sporeforming capacity and Gram category (G+/G-) are the most influential factors affecting inactivation. For spores D_{10} (kGy) was at 4.27 (±1.38) kGy and for vegetative cells 1.83 (±0.84) kGy. Estimated parameters from the meta-analysis of inactivation in spices and herbs were compared against meta-analyses conducted in other foods. Results show that irradiation inactivation of microbes in spices and herbs is less effective than in other foods while thermal inactivation of spores does not appear to differ.

Detailed experimental quantification for modelling the irradiation inactivation in paprika spiked with *Salmonella* Infantis and *Bacillus cereus* was performed. The irradiation studies conducted showed that at irradiative dose of 10 kGy, >6 log reduction was obtained for *Salmonella* Infantis and 4.5 log for *Bacillus cereus* spores. Irradiation parameter D_{10} (kGy) estimated from linear regression was derived as 1.33 and 2.27 for *Salmonella* Infantis and *Bacillus cereus* spores, respectively. Results show that inactivation of these microorganisms in dried spices can be predicted from the parameters estimated from the meta-analysis on spices and herbs.

Regarding survival in dried herbs and spices during storage, published studies were collected and data were analysed. The most influential factors were sporeforming capacity and temperature for the sporeformers. The estimated D value from the meta-analysis for sporeformers was 2.2 years while for cells of non-sporeformers it was 0.5 years. Data variability on cells of non-sporeformers was very large and did not allow accurate predictions (slopes close to zero). However, these results concur with another major meta-analysis and indicate that pathogens in spices and dry herbs at a temperature of 20°C can be very persistent over time. Findings of the meta-analysis were in accordance with the results of tenacity studies performed within the SPICED project.

The ALOP/FSO approach was used to investigate the concentration/frequency of *Salmonella* spp., *Listeria monocytogenes* and *Bacillus cereus* in spices (different scenarios), namely the FSO that contributes to the ALOP. By taking into account the mortality rates of the pathogens and assuming that only one death occurs from consumption of contaminated spices and herbs every year and every 100,000,000 people, an ALOP could be estimated. The result was an assumed ALOP for each of the pathogens in the scenarios (e.g. *Salmonella* spp. of 128 illnesses per year per 100,000,000 people). In addition, by taking into account the average daily consumption of paprika 0.11 g and the exponential model, the FSO was estimated for each of the organisms.

Finally, all data on initial level, inactivation and survival were combined to relate them to an FSO and estimated public health effects. This has been done both for the average condition, but also including variability.

By using this approach it is possible to predict survival of food-borne pathogens in spices and herbs and when appropriate, to evaluate the control of their numbers and reduce the risk of getting ill by those pathogens.

<u>Status quo of the spice and herb supply chain and detecting vulnerable points</u>

To identify vulnerable points in the spice and herb chains, selected chains were analysed. Trade network analyses were performed by creating corresponding food balance sheets for pepper, paprika, vanilla, cinnamon, nutmeg and saffron. For that, trade data (export, import) were used with respect to various participants of the trade chain (forwarder, destination country, bulk and quantitative splitting) were considered. Detailed turnover data were collected for crushed or ground fruit of the genus *Capsicum* or pimento (HS code 09042090), pepper of the genus *Piper*, not crushed, not ground (HS code 09041100) and pepper of the genus *Piper* crushed or ground (HS code 09041200) based on the Eurostat database, as well as further European and non-European databases. Data collection and interpretation were subsequently extended to other spices with available statistical data (cloves, ginger, nutmeg – mace, cardamom, vanilla, cinnamon). Data matrices from data between 2004 and 2011, which facilitated analysis of the dynamics of turnover changes, and production data of paprika and pepper from the production statistics of FAOSTAT that showed the relevance of the EU in the world turnovers, allowed detailed network analysis. The spice (category) used in the largest quantity in the EU is *Capsicum* and pimento (from 49 countries), the second is pepper (from 47 countries) and the one used in the least amount vanilla. Except of nutmeg and cloves the production and the EU import increased for all analysed spices, with the strongest increase observed for ginger (e.g. the EU import from 2002 to 2011 increased 2.55 times according to the Eurostat data). However, actual increases are difficult to judge due to the expansion of the EU between 2004 and 2007. On the other hand, it is evident that the spices supply chain has become increasingly complex during the last decade.

Within the scope of the trade analysis performed within SPICED, network connection characteristics of countries involved in international spice trade (brokerage role, eigenvector centrality, authority centrality, betweenness centrality, in-degree centrality, out-degree centrality) as well as descriptors of the entire trade network (density, clustering, diffusion, efficiency, centralisation, average betweenness) were assessed using an object-relationship-attribute (ORA) model. Based on the model, Spain, Germany and the Netherlands play the most influential brokerage role; Germany, Great Britain and the Netherlands have the largest authority centrality; Germany, the Netherlands and Great Britain have the greatest in-degree centrality; and Spain has the largest out-degree centrality. The six network indicators made it possible to monitor changes over time.

<u>Spice and herb production characteristics and detecting vulnerable points</u>

To assess spice production characteristics and to identify vulnerable points, spice paprika production was chosen as a model. Thus, paprika production from field cultivation to packaged end product was analysed for its beneficial components (bioactive substances), hazard-related organic micro-contaminants of agricultural origin (pesticide residues) and microbial contamination in terms of the capacity to produce mycotoxins. For these purposes, research has been carried out in several main directions: i) chemical and microbiological analysis of spice paprika samples with known origin obtained from producers/manufacturers and commercial sources; ii) analysis of paprika cultivation under various agrotechnological conditions at a model level; and iii) assessment of spice paprika cultivation and production using samples from paprika growers in Hungary and a major spice paprika manufacturer.

Microbiological and chemical analyses of spice paprika samples from EU and non-EU countries (including Hungarian, other European, Chinese, and South American samples) were carried out. As for the microbial loads of paprika, no substantial differences among the paprika samples originating from different countries within the EU market were found. Similarly, no considerable differences among the paprika samples from domestic, foreign, industry or primary producers were identified, but mould contamination was found to be systematically lower in all samples than analysis results from earlier years. In general, five species, namely *Bacillus cereus*, *Bacillus licheniformis*, *Bacillus mycoides*, *Bacillus subtilis*, and an unidentified *Bacillus* sp. represented 85% of the dominant bacteria in the samples analysed, independently of the origin. The black aspergilli and *Aspergillus flavus* were among the most commonly occurring food spoilage moulds on paprika. After total genomic DNA isolation, 16S rDNA specific PCR reactions were performed to identify non-culturable bacterial contaminants, and several species (including *Pseudomonas* spp. and *Escherichia coli*) were identified. As mycotoxin contaminants in these paprika samples, determined by immuno analytical approaches (ELISA, immunosensor) and liquid chromatographic method (HPLC), ochratoxin was found in 38% of the samples, in two cases (from Spain and China) above the legally permitted level. Aflatoxins were identified in 25% of the samples and in three cases (from Peru and Serbia) above the legal limit. Among pesticide residues, contamination rates of these samples were usually under the limit of detection (LOD), but certain pesticide residues (e.g. chlorpyrifos) could be detected in some cases.

Model cultivation experiments have been carried out to assess the impact of a heavy use of pesticides on paprika product quality. Formulated pesticides of four active ingredients (pirimicarb, chlorpyrifos, penconazole and cypermethrin) were applied at 0.2-, 1- and 5-fold rates of recommended doses. Paprika fruit samples contained pesticide residues in occasional trace amounts in produce treated at 0.2- and 1-fold doses, whereas significantly higher levels were determined for paprika treated with 5-fold doses (e.g. 12.5–174 ng/g of chlorpyrifos). The content of bioactive components (carotenoids) was found to be slightly compromised. Paprika cultivation fields were sampled for both crop (plant parts and paprika fruit) and environmental (soil, surface water) samples under various agricultural cultivation conditions (ecological farming, intensive agriculture) in Hungary. Soil samples originated from organic farming were found not to contain pesticide residues above the LOD, while in other soil samples one or more pesticide active ingredients (trifluralin, tefluthrin, chlorpyrifos and DDT with their metabolites, as well as traces of diazinon, atrazine and metolachlor in some cases) were detected. Paprika fruits were found not to contain detectable residues of these pesticides.

The composition and contamination status of spice paprika along its processing line was investigated in a case study with samples obtained at various points of the production technology. External factors, such as cultivation features, harvest procedures, storage duration and temperature, processing protocols, conditions exacerbating water loss, post-ripening were found to affect the quality of the commodity. Drying and milling were identified as most critical steps of spice paprika processing: the quality, nutritive value and storage stability depends, to a high extent, on the conditions at which these technological steps are performed. Accordingly, in the production technology, there are three critical control points (CCPs) in the production line: at the drying step, at the microbial decontamination stage, and for the compositional and contamination checking of imported half-products.

Furthermore, EU regulations for export/import in the spice and herb chain were investigated. Several market studies per spice/herb showed that vulnerability is spice dependent and is dependent on if further processing is conducted inside the EU, for instance before an EU country exports it again to neighbouring countries. Afterwards, a gross list of potential factors that may influence/have an effect on vulnerability in the spices and herbs chain, as well as points in the chains at which these factors have their effect (vulnerable points) were provided to task partners. A literature review on the import and export characteristics for specific spices and herbs was performed to gain more insight on the vulnerable points (e.g. fraud). Subsequently, a food fraud vulnerability assessment tool was offered to spice companies to visualise the likelihood of food fraud occurring in the company. Several quality assurance managers of spice companies ($n \approx 8$) were personally interviewed to gain more insight about fraud per spice and in the supply chain. This includes importers, business to business, and business to consumer companies. Actors further along in the chain, namely, retail organisations were contacted to discuss the impact of authenticity issues.

Standard inclusion rates of spices and herbs in food and exposure assessment for selected agents in risk products

To gain insight into consumption behaviour regarding spices and herbs, a targeted questionnaire was developed. This questionnaire addressed the assessment of the amounts of eleven selected herbs and spices (paprika/chilli, black pepper, nutmeg, cinnamon/cassis, allspice (pimento), saffron, vanilla, parsley, oregano leaves, basil leaves (sweet), thyme leaves) consumed individually at home. Data collection was carried out in all project partner countries resulting in the following numbers of individual surveys: Austria 276, Germany 281, Hungary 318, Ireland 220, Latvia 259, Slovakia 246, and the Netherlands 266. These numbers were sufficient for statistical analysis. On the basis of the detailed evaluation of the data obtained in these surveys, the socio-demographic background of the subjects, their frequency of food preparation at home, the most frequently self-consumed/purchased herbs and spices, the cultivation and purchasing format of herbs and spices, consumption frequency of national and international dishes, as well as habits and level of knowledge regarding herbs and spices were analysed. On the basis of the questionnaire survey, the spices 'pepper' and 'paprika' and the herbs 'parsley' and 'basil' are the most frequently consumed. 'Parsley' is often used in a fresh form (e.g. cultivated at home, purchased in a pot, or as fresh/frozen produce), while 'paprika' and 'pepper' are mainly used in a dried form. Consumption of 'saffron' and 'allspice' are the lowest recorded. Popularity of Italian and national style dishes – with their special herbs and spices – are quite high in the analysed countries. According to the survey results, national particularities can be identified as, for example: Hungarian participants strongly prefer spicy dishes, while Slovakians do not; members of the Latvian group noted that they like to add some season to their dishes at the table; Austrian respondents like to spice their dishes with fresh spices; and Irish consumers are highly interested in gastronomic topic (TV programmes, articles). Knowledge of 'herb and spice' food safety aspects is not satisfying; thus, the consumers' education in this topic is necessary. Furthermore results of the survey draw attention to the outstanding importance for analysing food safety aspects in 'paprika' and 'pepper'.

Based on data from above questionnaire and of the EFSA Comprehensive European Food Consumption Database, standard inclusion rates of spices and herbs in foodstuffs were calculated. For that, the frequency of the spice/herb used, habits regarding 'ready to eat' dishes, as well as recipes of the most frequently consumed dishes on, of which were based on a targeted analysis of the data obtained in the above questionnaire were utilised. According to the results, herbs and spices represent a minute amount of the recipes. Green herb consumption (mainly basil and oregano) was mentioned in the case of Italian style dishes, which are consumed all over the world. However, the most frequently consumed dishes differ among the considered countries. This needs to be taken into consideration during the exposure assessment of herbs and spices. Exposure assessment for selected agents in risk products was carried out based on standard inclusion rates and infectious doses.

Evaluation of on-site sampling technologies and statistics

Monitoring hazards require, besides proper detection methods efficient sampling. Within the SPICED project, a simulation model was developed in the framework of a milestone report comparing different sampling strategies with respect to their ability to detect a contamination in a spice or herb lot. The objective was to gain more insight into applied sampling strategies and to propose a sampling protocol for spices and herbs. The sampling approaches compared were simple random sampling (SRS), stratified random sampling, (STRS), and systematic sampling (SS). These sampling strategies were evaluated considering the effect of heterogeneity of contaminant distribution, and the overall contamination. Results indicate the best sampling strategy with number of samples. Also, a simulation study on the cost-effectiveness of sampling was performed. From this, the most cost-effective sampling strategy can be selected. This model was developed and validated using information from industry partners. Also, sampling under real conditions was organised to acquire information about the procedure from product to laboratory sample. From a 287 kg batch, every 14 kg one sample was taken. In total 20 samples, à 50 g, were sampled and analysed for *Salmonella* and for the presence and quantity of Enterobacteriaceae, sporeformers, and total viable count.

Conclusion

Within all tasks of WP 2, important achievements and knowledge gains were made. The outcomes of WP 2 were published in several peer-reviewed publications. Further publications are in progress, covering for example the chain and trade network analyses, the consumption survey, the decontamination of paprika, and the effective sampling strategy to detect a chemical/microbial contamination within a lot of spices and herbs.

The **WP 3 "Biological Hazards"** aimed at the characterisation of the properties of biological hazardous contaminants and their reliable and standardised detection in spice and herb matrices. Spices and herbs might be present in almost every processed food and are hence reaching a wide distribution although they are only minor food ingredients. In addition, spices and herbs can contain various microorganisms, like *Salmonella* spp., *Bacillus* spp., *Clostridium* spp., which can be a risk factor for consumer's health. This becomes particularly obvious as spices have been associated with various food-borne outbreaks. At least 22 outbreaks linked to microbial contaminated spices have been reported within Europe since 1973 and it must be assumed that many outbreaks remain unreported. Therefore, spices and herbs are of interest when one considers food safety matters related to biological hazards.

Within WP 3, extensive work was conducted on i) improving and developing inoculation methods for artificial spiking experiments and analysing the survival of biological agents in different spice/herb matrices, ii) establishing a database on diagnostic methods, iii) adapting and optimising sample preparation and detection, and iv) differentiating living and non-living bacteria in spice and herb matrices. Moreover, v) rapid qualitative on-site detection/screening methods and vi) quantitative high-throughput detection methods for biological contaminants in spices and herbs were developed and evaluated and vii) a ring trial for standardisation and harmonisation of diagnostics was conducted.

Conduction of tenacity/stability studies using spiked spices and herbs

Despite the fact that various microorganisms have been detected in naturally contaminated spices and herbs, it can become surprisingly difficult to artificially contaminate low-moisture products. Therefore, several spiking techniques were tested, also taking possible natural contamination pathways into account. In total, eleven inoculation methods for artificial spiking of spices and herbs with biological contaminants were improved and/or developed by the SPICED consortium. The spiking methods tested included i) addition of dried agents, ii) addition of agents in liquids by direct contamination of the matrices, iii) indirect contamination by using carrier materials that were added to the matrices after drying, and iv) addition to ready-to-eat food. Significant differences in the survival time of vegetative cells and endospores were found due to the spiking technique used. Further, it could be demonstrated that the bacterial species used, the spice and herb matrices themselves, and the temperature at which storage took place can have a significant impact on the survival time of the bacterial contaminants in the various spice and herb matrices. Tenacity/stability studies were conducted for almost all combinations of agents and matrices in the focus of SPICED. The results will be published in peer-reviewed journals (manuscripts are under preparation).

Development of a database containing diagnostic methods

Qualitative and quantitative detection methods including sample preparation methods for various combinations of biological hazards and spice/herb matrices were tested and optimised by the SPICED project consortium. Available detection methods were found to be limited for several agent–matrix combinations. The heterogeneity of spices and herbs is very high (more than 400 spices and herbs are available in Europe) and several of the matrices are difficult to analyse due to interfering substances. However, sometimes slight changes in the protocol can improve the LOD or change the result from false negative into a correct positive. One of these difficult agent–matrix combinations was selected for validation of an improved sample preparation and agent detection method in a ring trial as described below. All diagnostic methods used within the SPICED consortium were collected in a database to generate a harmonised knowledge tool for possible sample preparation and detection strategies. The database is currently only available for the SPICED consortium; nevertheless, several opportunities to make it available to the public are under discussion.

Adaptation and optimisation of sample preparation and detection methods, including standardisation and harmonisation of diagnostics by a ring trial

Within the SPICED consortium a special focus on sample preparation and detection methods was set on the following biological hazards: *Bacillus cereus*, *Brucella microti*, *Clostridium perfringens*, *Salmonella enterica*, *Staphylococcus aureus* and its enterotoxin B (SEB) as well as ricin. The various interfering substances of spices and herbs can lead to incorrect results when using culture-based and/or culture-independent methods. Thus, improvements and adaptions of standard methods (e.g. ISO methods) were required. In the following, results obtained for *Salmonella* Oranienburg (culture-based methods) and *Staphylococcus aureus* (comparison of culture-based and culture-independent methods) are outlined. Detailed data on *Salmonella* Oranienburg and data on further biological agents, which were achieved within SPICED and provided in milestones and deliverables, will be published in peer-reviewed journals.

A strong bactericidal effect on *Salmonella* Oranienburg was detected for cinnamon and oregano, while allspice and thyme showed temporal inhibitions only. Thus, ISO 6579:2002/Amd.1:2007 was modified according to ISO 6887-4:2012, in terms of an increased initial dilution of 1:20 (w/w) and application of 0.5% K_2SO_3 with the buffered peptone water (BPW). This optimised method had a higher sensitivity with various matrices. Additionally, the 3M Molecular Detection System (MDS) was used for comparison of the LOD with the ISO method. The ISO showed an LOD of <5 colony forming units (cfu)/25 g, while for the MDS 40 cfu/reaction were required, corresponding to about 10^3 cfu/ml BPW. However, if an appropriate enrichment takes place, these LODs are satisfactory. Besides cinnamon, the most challenging matrix for *Salmonella* Oranienburg was oregano, still showing high antimicrobial activity at a dilution of 1:20 (w/w) (25 g + 475 g BPW).

The modified ISO method was successfully used for the detection of *Salmonella* Oranienburg in cinnamon (25 g) within a ring trial performed among seven SPICED project partners. In a pre-trial, a stability testing of the inoculum was performed where samples were sent from one partner to another and back. Then they were stored at 5 ±3°C (according to ISO 7218:2007) until the maximal storage duration of three weeks was achieved. Summed up, the transport and storage had no remarkable effect on recovery of *Salmonella* Oranienburg. In January and February 2016 the ring trial was conducted where in total ten sets of samples were provided. One set consisted of 15 samples (three concentrations *Salmonella* Oranienburg: 50 cfu/bag, 200 cfu/bag and absence of *Salmonella*, each as five replicates), plus a positive and a negative control. All partners used the suggested modified ISO method. From them, one has additionally used the common ISO and an in-house method, and another one also the common ISO method. All used the culture-based detection, while four partners also used a PCR-based detection. In total, 255 results were determined. Summed up, the culture-based detection after the modified ISO enrichment resulted in 95% correct results, while the PCR-based approach was slightly worse with 92% correct results. However, all partners and methods were able to detect 200 cfu *Salmonella* per bag, a concentration, which is stated as very low by accredited and certified ring trial conductors. In addition, all results with the in-house method, where a washing and shaking step was used prior the enrichment, were correct. Interestingly, the modified ISO led to a highly significant improvement of the *Salmonella* detection because not a single sample was positively detected with the common ISO 6579 due to antimicrobial effects of cinnamon. However, the latter method is probably still used in several laboratories in Europe, or even around the world, and thus could lead to several false-negative results, bearing a high risk for humans. To conclude, the results of the participants within the conducted ring trial with the suggested modified ISO method were satisfactory to excellent.

Further, within SPICED, variants of ISO 6888-1:1999 and ISO 6888-3:2003 methods for the detection of *Staphylococcus aureus* were evaluated and improved for their application to conduct analysis in spices and herbs. Improvement was achieved by washing of the sample to remove compounds interfering with analysis and in the use of PCR for final detection, instead of plating on Baird Parker agar. This improved sensitivity at high backgrounds and saved time. The evaluation of the method variants was based on determination of the LOD using a series of artificially contaminated spices (allspice, black pepper, cinnamon, nutmeg, paprika, vanilla) and herbs (basil, oregano, parsley, thyme). The method without enrichment, ISO 6888-1:1999, resulted in LODs of 10^3–10^5 cfu/g with no positive effect of washing the sample or use of PCR for final detection. The method with enrichment, ISO 6888-3:2003, had an LOD of 10^0 cfu/g for basil, black pepper, paprika, and parsley. If the washing step was added and PCR was used for final detection, an LOD of 10^0 cfu/g was also determined for cinnamon, nutmeg and vanilla, and an LOD of 10^1 cfu/g was determined for allspice. These results were published in a peer-reviewed journal (Cabicarova et al. 2016, http://link.springer.com/article/10.1007%2Fs12161-015-0379-0). For oregano and thyme, which strongly inhibit the growth of *Staphylococcus aureus*, an alternative enrichment-independent method based on direct DNA extraction coupled to real-time PCR was subsequently developed as an advantageous alternative. Further publications on optimising sample preparation and biological hazard detection are in progress.

Differentiation of living and non-living bacteria

However, direct DNA-based methods like PCR are usually unable to distinguish between live and dead bacterial cells. Therefore, such methods may suffer from a level of false positives, in particular if the food samples contain devitalised bacterial cells e.g. after decontamination. Because of that, in the SPICED project, the particular states of viable but non-culturable cells as well as living and non-living cells were taken into account for the development of alternative culture-independent methods. The SPICED project partners used three different approaches including a PCR-based method, fluorescence microscopy and flow cytometry. Here, a closer look is taken on the work conducted using the last mentioned approach.

Flow cytometry (FCM) analysis was employed to detect and enumerate viable *Escherichia coli* cells in cinnamon, basil, oregano, and nutmeg. In addition to fluorescent dyes, an anti-*Escherichia coli* antibody was employed to specifically label *Escherichia coli* cells. The sample clean-up procedure and the gating strategy resulted in the recovery and separation of live/intact *Escherichia coli* cells from these three matrix' particles when spiked by 10^4–10^7 cfu/g of *Escherichia coli*. The recovery of the bacteria was confirmed by enumeration of events in the live *Escherichia coli* population of FCM and by traditional plate count methods. Also on this task, peer-reviewed publications are planned.

Development and evaluation of rapid qualitative on-site detection methods and of quantitative high-throughput detection methods

In addition, the SPICED consortium conducted research on the development and evaluation of rapid qualitative on-site detection/screening methods as well as of quantitative high-throughput detection methods for biological contaminants. Various combinations of different agents, matrices, and techniques were investigated. In the following, two techniques optimised for rapid quantitative high-throughput analyses are exemplarily described in more detail. Further data obtained will be published in peer-reviewed journals.

For example, a culture-independent method was developed for quantification of pathogenic bacteria in spices and herbs. The method is based on DNA extraction using cetyltrimethylammonium bromide (CTAB) and on real-time polymerase chain reaction (PCR). When evaluated with spices (black pepper, paprika) and herbs (oregano, parsley) that were artificially contaminated with *Staphylococcus aureus, Salmonella enterica* or *Escherichia coli*, the method demonstrated quantitative response with linear calibration lines and quantification limits of 10^2–10^4 cfu/g. The developed method is suitable for rapid microbiological analysis of spices and herbs, taking 8–9 h (Minarovičová *et al.*, submitted).

In addition, based on the FCM analysis described above, a fluorescence activated cell sorting (FACS) detection method was established for cinnamon, black pepper, basil and nutmeg samples spiked with *Escherichia coli*. Events from distinguished (live, dead and matrix) sub-populations of spice/herb samples spiked with *Escherichia coli* were sorted from each region and plated on plate count agar and tryptone bile X-glucuronide (TBX) agar. As expected, no growth was observed on plates with events sorted from dead and matrix regions. Colony counts on both agars were in agreement with the number of events sorted from the live regions (10^5 events). Furthermore, the distinguished populations of live *Escherichia coli* labelled with an anti-*Escherichia coli* antibody in each matrix were sorted. The sorted number of events (10^5) reflected the colony counts on both agars. Thus, FACS can serve as a technique to both quantitatively and specifically detect viable microorganisms in spice and herb matrices.

Conclusion

In conclusion it can be stated that the heterogeneous matrices of spices and herbs can bring microbiological, molecular biological and immunological techniques to their limits and therefore, require adaptation and optimisation as performed within SPICED. Some microorganisms can survive in spices and herbs for several weeks to months and even years, whereas the tenacity is particularly dependent on the species and individual strain. Additionally, tenacity depends also on the herb/spice matrix, on the way of contamination and on the cell type in case of sporeformers. The great amount of reliable data obtained by improving diagnostics and conducting tenacity studies underlines the scientific quality of and the great team work within the SPICED consortium.

The **WP 4 "Chemical Hazards"** aimed at developing a rapid and cost-efficient set of methodologies for the detection of natural and accidental as well as of deliberate contaminations of spices and herbs with chemical agents.

Review of the application of fingerprinting methods for spices and detection of chemical hazards

At the beginning of the SPICED project, the project partners of WP 4 reviewed scientific articles to get an overview of the research on the determination of authenticity and on adulterations in the selected spices and herbs. Both targeted and untargeted analytical methods were studied and the required pre-treatment steps were evaluated. In parallel, research on fingerprinting techniques was conducted and assessed. So called "fingerprinting techniques" are non-targeted approaches which are typically based on high-throughput sample screening using a spectroscopic or spectrometric method with the purpose of differentiating or classifying of samples. To recognise patterns in the acquired data, multivariate statistics were used. A database was prepared that includes information about fingerprinting techniques (used for authentication processes and detection of abnormalities and chemical hazards) and the corresponding chemometrics. In addition, a report was composed with an overall comparison of fingerprinting methods and chemical hazards. Furthermore, more than 60 scientific publications related to the (multi-)targeted detection and quantification of chemical elements and organic contaminants (mycotoxins, pesticides, colourings) in spices and herbs were evaluated. The main focus was on scientific papers, which used chemometric methods for the assessment of scientific data. This database contributed to the final selection of instrumental parameters for the analysis of spices and herbs within the SPICED project.

Development and evaluation of fingerprinting methods to detect chemical hazards in spices

In the next step, standard operating procedures (SOPs) for sampling, packaging and storage of spices/herbs were adopted and harmonised and analytical procedures were developed enabling high-throughput authentication of spices and herbs especially with respect to chemical hazards. In view of the applicability to analyse various authentic as well as adulterated spices and herbs, different sample preparation strategies for non-targeted analysis using spectroscopic and spectrometric techniques such as proton-transfer-reaction mass spectrometry (PTR-MS), high performance liquid chromatography-high resolution mass spectrometry (HPLC-HRMS), nuclear magnetic resonance spectroscopy (NMR), and Fourier transform infrared spectroscopy (FTIR) were tested and optimised. In addition, procedures for detection and quantification of chemical elements, mycotoxins, pesticides, and organic dyes in spices and herbs using different analytical methods (inductively coupled plasma mass spectrometry (ICP-MS), HPLC-HRMS and HPLC-MS/MS) were developed. The SOPs were summarised in a report. A method for the reliable screening of mycotoxins and pesticide residues in paprika based on ultra-high performance liquid chromatography coupled to high resolution Orbitrap mass spectrometry (UHPLC-Orbitrap-HRMS) was published in a peer-reviewed journal (Reinholds *et al*. 2015a, http://dx.doi.org/10.1016/j.foodcont.2015.09.008).

Another focus was set on the evaluation of several chemometric techniques for analysis of acquired spectroscopic and spectrometric data. Relevant scientific articles were identified, collected, reviewed and listed in a dedicated database. The database contains information on analytical techniques and chemometric approaches (including data pre-processing steps and validation procedures) for the analysis of different types of matrices found in literature. Further, a literature review is available, in which status quo of the most suitable chemometric methods for chemical hazard detection in spices and herbs including theoretical aspects and potential application are described (Reinholds *et al.* 2015b, http://dx.doi.org/doi:10.1016/j.jfca.2015.05.004).

The SOPs have been applied for the analysis of condiment matrices with spectroscopic and spectrometric techniques. Accordingly, the following spice/herb matrices have been used for the characterisation of contaminants, namely mycotoxins, pesticide residues, elements including heavy metals (Reinholds *et al.* 2016, www.tandfonline.com/doi/abs/10.1080/19393210.2016.1210244), and artificial colorants, and for non-targeted analysis: paprika/chilli, black pepper, nutmeg, basil, oregano, and thyme.

Setting standards for natural variation in authentic spices and development of a database with authentic material

The database covering the natural variation of authentic spices and artificially adulterated test samples was formed by MS Excel based spreadsheets developed separately due to the large data sets for each of the authentic spices and herbs (50 samples per matrix, in total 300 spice and herb samples). The database was created in a form of 6 compressed Excel files of authentic data sets. These files were developed in order to gain an easy survey of needed information on different batch files, methods of analysis and to facilitate the data assessment. In a separate spreadsheet, the original information of meta data (including information on batch files, sample origin, harvest, production, and storage conditions) is presented for each spice/herb. In the other spreadsheets, the data sets obtained with each method are collected. Besides the non-targeted analysis of the spice and herb samples using PTR-MS, direct injection mass spectrometry (DIMS), FTIR, and NMR, the samples were analysed for 11 mycotoxins, 134 pesticides, and 4 heavy metals using HPLC-Orbitrap-HRMS, HPLC-QqQ-MS/MS and ICP-MS. Mycotoxins were detected in 4%, 10%, and 30% of all nutmeg, basil, and thyme samples, respectively. The residues of 24 pesticides were detected in 59% of the analysed condiments. The maximum residue levels for pesticides were exceeded in 10% of oregano and 46% of thyme samples. In addition, the risk assessment of heavy metals was performed, indicating daily intake levels far below the tolerable intake levels.

Investigating spices spiked with adulterations as well as evaluation and validation of chemometric techniques to detect anomalies

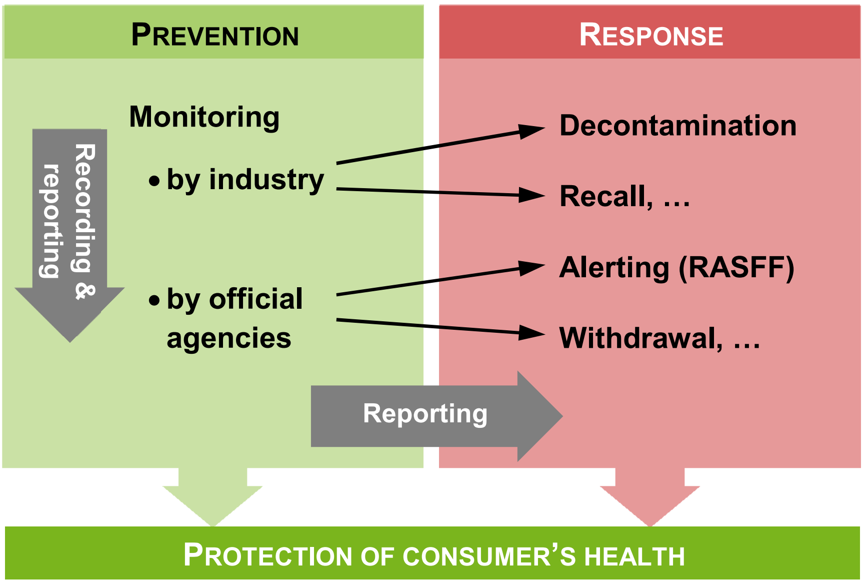
After analysing the baseline material (non-adulterated spice/herb samples provided by the industry partner), a spiking study of two spices (nutmeg and chilli paprika) and one herb matrix (oregano) was carried out: Nutmeg was adulterated with nutmeg spent, chilli paprika with the forbidden colourants Sudan I and Sudan IV, and oregano with olive leaves. This experimental design was developed allowing comparison of methodology for product-own and product-foreign adulterations. With respect to the above mentioned literature review, relevant chemometric methods were identified to detect adulterations of spices/herbs and several pattern recognition as well as classification techniques were tested. Finally, it was decided to perform the data analysis with one class classification (OCC). OCC uses the baseline samples (non-adulterated samples) to learn "normal" variation. Several model algorithms were tested, such as: CSimca, kNN, PCAresid, and SVM. Models were created based on the baseline samples, validated and challenged with artificially adulterated test samples. A report on the results of this study was prepared containing detailed information on the developed models (model parameters), the performance and success rates of the models.

Conclusion

In conclusion, it can be stated that fingerprinting techniques in combination with chemometrics are a useful tool for the purpose of ensuring the authenticity of spices/herbs. Especially the applications of analytical methods are promising, that can detect addition of adulterant material in non-targeted fashion. The non-targeted methods are expected to be able to detect a variety of deviations from normal (authentic) samples, and especially for those methods that are considered as high-throughput methods, this could mean that they are a valuable contribution to protect the authenticity of spices and herbs throughout the supply chains.

Nevertheless, each technique has its own advantages and drawbacks (e.g. investment costs or detection capabilities) thus that complementary approaches might be indicated for most comprehensive confidence in analyses.

Within **WP 5 "Prevention and Response"** focus was given on evaluating the possibilities and limitations of available mechanisms within the spice and herb supply chains to improve prevention of and response to food-borne incidents caused by biological and chemical contaminations. In terms of food safety, food-borne incidents caused by biological and chemical contaminations should be avoided or recognised as soon as possible to prevent a further spread. Therefore, mechanisms which provide data on hazards in the food chain are needed. Within WP 5 i) alerting mechanisms were analysed including an evaluation of the possibilities and limitations of the European Rapid Alert System for Feed and Food (RASFF), ii) reporting mechanisms (including data record systems) according to law and private quality management systems have been investigated and best practice examples were established, and iii) recommendations for an effective monitoring system for most relevant hazards in the spices and herbs chains were defined. Moreover, iv) a guideline for differentiation between natural and intentional incidents was developed. In addition, v) a database on decontamination methods including legal aspects was established, and vi) means and techniques for decontamination of production plants and spices and herbs were evaluated. Finally, vii) the rapid implementation of new detection methods on the market was investigated. The *Escherichia coli* O104:H4 food-borne outbreak in Germany 2011 showed that it is of importance that reliable detection of pathogens can be ensured even if methods that are not established within laboratories and/or no standardised protocol (e.g. ISO, national law) have to be used.



Alerting mechanisms – Possibilities and limitations of RASFF

The overview of possibilities and limitations of RASFF established within the SPICED project aimed to evaluate the utility of RASFF for early identification of food safety issues in the spice and herb chains, including known hazards and emerging hazards. As part of this, the possibilities and limitations of RASFF as an early identification tool were elucidated. The RASFF data were analysed for food safety hazards including chemical related hazards, microbial pathogens, and food poisoning notifications. Based on this work, a joint scientific publication entitled "European alerting and monitoring data as inputs for the risk assessment of microbiological and chemical hazards in spices and herbs" was published in the journal Food Control (Banach *et al.* 2016, http://dx.doi.org/10.1016/j.foodcont.2016.04.010). Additionally, a work-flow of the RASFF notifications related to the product category "herbs and spices", was written and can be used for the open source software KNIME.

Reporting mechanisms (including data record systems) according to law and private management standards

Numerous public and private standards are available to ensure food safety and protect the consumer's health. An overview report was prepared that provides information on the current EU food law, public and private food standards, and private food safety management standards as well as on the recording and reporting mechanisms for ensuring food safety and enabling food traceability. General information was obtained by a literature/internet search. In addition, a survey was conducted to gain data from the culinary herb/spice industry on (i) applied quality/food safety management standards and product safety standards, (ii) information collected (including product testing) and reported along the culinary herb/spice commodity chain, and (iv) the manner of data reporting. Based on the literature and survey data, suggestions on "best practice" recommendations on recording and reporting for culinary herb/spice handling businesses in non-EU member countries were drafted as basis for discussion. Moreover, recommendations on a standard electronic record system have been developed. Based on the work, two scientific publications on public and private standards for dried culinary herbs and spices are published in Food Control (Schaarschmidt 2016, http://dx.doi.org/10.1016/j.foodcont.2016.06.004; Schaarschmidt *et al.* 2016, http://dx.doi.org/10.1016/j.foodcont.2016.06.003); another peer-reviewed publication is in progress.

In addition, national legal and authority regulations of reporting and recording food contamination were surveyed for spice paprika production and quality management from field cultivation to packaged end product in Hungary as an example. The survey aimed to assess i) in what context or extent national regulations and systems are identical to or may differ from the practice of the EU RASFF; ii) how the food safety system (organisation structure) operate(d) before and after joining the EU; iii) if there are any changes in the food legislation and authorisation system, as well as organisation of food contamination reports and registration; and iv) how the responsibility levels may have changed. The monitoring systems were also overviewed, covering the range of organisation responsible for annual and seasonal sampling regime plans and their execution; food product group to be included in the sampling regimes; possible changes in the sampling protocols and in sampling frequency; institutions carrying out the analyses; public access possibilities to sampling regimes and results; the national organisation responsible for reporting the problematic samples to the RASFF network; and possible improvements since joining the RASFF system. Within the reported period, data of the RASFF and governmental monitoring systems were evaluated for their suitability as alerting and early warning systems within the EU, and points of vulnerability have been described from the aspects of accidental and deliberate contamination. The efficacy of the concerted action of producers' quality management practices with corresponding government measures were illustrated, and intensified interaction and cooperation of the private and government sectors were urged.

Recommendations for an effective monitoring system for most relevant hazards in the spice and herb chain

Governmental monitoring systems for the spices and herbs chains (EU, The Netherlands, and Germany) were evaluated and monitoring systems for chemical hazards in these countries were reviewed. Legislation on sampling, analyses, and legal limits for food safety hazards, including chemical hazards and fraud/adulteration were investigated. Four companies from the Dutch spice and herb industry were interviewed about these hazards and asked to provide input on their supply chain. Data on information on existing industry monitoring systems in general and of one company in detail were made available by industry partners. Based on these data and input from WP 2, an optimisation model for effective monitoring of chemical hazards in the supply chain, using Bayesian modelling was developed. The model focused on sampling at the national level (i.e. by a Member State), and the Dutch situation served as an example. The model covers the entire spices/herbs chain and control points in this chain (border control/import, company, market, and customer). This helped to optimise monitoring over the chain with the aim to be used by supply chain actors. It covers the most relevant chemical hazards, products, and origins and utilises volume/trade data and contamination data. Results were described in a project deliverable and presented on scientific conferences. Two scientific publications related to task topics are currently under preparation.

Guidelines for differentiation between natural and intentional incidents

To support the differentiation between natural and intentional incidents, guidelines were developed by the SPICED consortium. So far, disease outbreaks caused by spices and herbs were usually assigned to natural condiment contaminations; currently, no intentional contaminations have been indicated. Consequently, the developed guidelines serve a broader purpose: as a supportive tool to analyse food-borne incidents to determine the likelihood of intentional contaminations via the food chain. In order to evaluate the incident and the appropriate control measures, one must highlight the origin of the source as well as the background related to the incident. In order to have authorities respond appropriately regarding the differentiation between natural and intentional incidents, the origin of the incident remains critical to determine. Likewise, the intentional nature of a contamination helps to support investigations in the detection of the incident source and may even help to prevent future attacks. Consequently, these guidelines are formulated based on a literature review of relevant differentiation criteria and by expert discussions within the project. In addition, the outlined indicators are evaluated based on a combination of such available information and insight.

Database on decontamination methods including legal aspects

Dried culinary herbs and spices can be contaminated with a high microbial load. To improve the quality and safety of these products, particularly when added to other foods without further microbial inactivation steps, decontamination measures can be applied. Within the WP 5, a database on microbial reduction methods was established to collect data on different technological approaches and their legal status and to support the evaluation of these methods. The treatment parameters considered cover approved technologies as well as potential technologies and new methods. Among those are irradiation with gamma, ultraviolet, infrared, and electron radiation, radiofrequency and microwave treatment, treatment with Bioptron lamp (polarised light of 480–3400 mm), steam treatment and fumigation. Moreover, combinations of different technologies and dosage effects were considered regarding the efficacy in the suppression of the microbial status as well as negative side effects on the product quality (e.g. colour). The decontamination database covers data obtained within the SPICED project, additional unpublished data obtained and provided by the project partners, and literature data.

Evaluation of available means and techniques for decontamination of production plants and spices

To further evaluate different technologies for their potential to reduce the microbial load of spices and dried herbs as well as for production facilities, experiments were performed within SPICED. Currently available decontamination methods for spices and herbs were particularly investigated and evaluated for spice paprika. As irradiation of spices has been approved by the EFSA as being a safe treatment method, it was compared to and assessed with other treatments on microbial contamination and quality of spices with regard to e.g. their colour and other sensory attributes. Steaming of spices is a proven method for microbial decontamination. Microwave and radio frequency treatments for this purpose are in their research phase, and were also assessed in the project. Thus, spice paprika samples were irradiated with ionising radiation (^{60}Co) with 1, 5, and 10 kGy, and results confirmed that the microbial contamination of spice paprika was effectively reduced by this method. Following irradiation treatment, the initially dominant microflora of *Bacillus spp.* (*Bacillus methylotrophicus*, *Bacillus pumilus*) gradually disappeared, and species less sensitive to irradiation (*Methylobacterium* spp., *Micrococcus* spp., *Microbacterium* spp.) came into view. Steam treatment (saturated dry steam, 108–125°C for 20–120 s) reduced mesophilic aerobic total bacterial count from 1.8×10^5 cfu/g to 6.0×10^2 cfu/g, and moulds from 1.3×10^2 cfu/g to under the detection limit. Yeasts, coliforms, *Escherichia coli* and Enterobacteriaceae could not be detected in the samples. Unlike irradiation, steaming had no selection effect on the surviving microflora. Microwave heating resulted in no relevant reduction of the mesophilic aerobic total bacterial count at the parameters studied; however, the colour of the paprika powder got darker and had a brownish character. Similarly, radio frequency treatment did not reduce the microbial load of the samples effectively even for the most severe treatment, yet the colour of all treated samples were significantly darker than the control, having a burnt character.

Rapid implementation of new detection methods on the market

Within the SPICED project, some general recommendations for a rapid implementation of new detection methods on the market have been established. The implementation of new analytical methods for detection of chemical and biological contamination of spices and herbs can be necessary if the established methods do not provide satisfactory analytical performance or speed, regarding specific combinations of contaminants and matrices. A need for new methods may appear during microbiological or chemical contamination incidents, in certain cases of fraud, threats or deliberate contamination. A particular need for rapid implementation of new methods may appear during crises or emergency situations. Implementation of new analytical methods can be taken as a component of the preparedness concept, as defined in various European countries.

Conclusion

In conclusion, it can be stated that various prevention and response mechanisms were considered under the special focus of spices and herbs. Therefore, various reporting and management mechanisms were investigated on the basis of legal regulations and private quality/safety management systems. Additionally, currently available decontamination methods for spices and herbs as well as for production facilities were performed and evaluated.

The potential impact and the main dissemination activities and exploitation of results

The SPICED project is the first that focuses on the diverse and complex food chains of spices and herbs taking major natural, accidental or deliberate contaminations into account. The project consortium covers partners from national food safety authorities and academia. Besides, the practical relevance of the project and its results has been ensured by integrating the culinary herb/spice industry into the consortium. The SPICED consortium comprises one of the biggest players of the spice and herb industry as project partner. Further, four integrated stakeholders from industry—including the European and the German spice associations—are part of the consortium.

The success of the project was further ensured by sufficient intra-consortium communication and regular assessment of the work progress and results. For that, various instruments were used, such as regular meetings and teleconferences with minutes, semi-annual reports, and appropriate databases provided to all project partners via a web-based internal communication tool.

In addition, project management including financial and administrative management was performed at a high level. A close and effective collaboration with the External Advisory Board and with the Beneficiaries has been ensured. Throughout the entire course of the SPICED project an excellent balance among relevant areas of expertise could be ensured and the work quality of participants was always on a high level. In addition, resources were minimised whenever possible and they have been used to achieve consistent progress, efficiently produce outputs, and ensure effectiveness according to the impact of an activity. Further, all deliverables have been submitted in time to the European Commission.

An intense exchange of knowledge with other EU and national projects took place within the entire period of the SPICED project. In addition, the scientific quality and the worldwide impact were ensured by increasing the knowledge on minor and dry food ingredients as a major aspect of food safety according to the "farm to fork" principle and strengthening the awareness of the major potential of minor components to contaminate a wide range of products in a largescale distribution area. One has to face several difficulties when standard detection methods are used for analyses in spice and herb matrices. Therefore, the scientific quality of the project was based on i) the use of cutting-edge technologies and innovative techniques, ii) the evaluation of impact factors on contamination and decontamination of spices and herbs, and iii) the improvement of sampling plans for statistical confidence.

The impact of the SPICED project was ensured by i) informing the general public on relevant food safety issues according to the motto “Spices and herbs – A risk-free taste experience?”, ii) increasing the awareness of policy makers regarding information on data limitations for the spices and herb chains, iii) providing the results about the *status quo* of contaminations in the spice and herb chains as well as on possibilities and limitations of diagnostic methods to the scientific community, and iv) actively involving spice and herb processing industry stakeholders in every step of the project as various data and knowledge exchanges expand the field of view of both, industry and research. Within SPICED, new strategies and tools related to the spice/herb chains have been developed and significant scientific and technical impacts have been produced, which have already been published or will soon be published.

SPICED has provided data on characterising production and supply chains on national, EU, and international levels. The analysis performed on the spice/herb production and supply chains and their vulnerable points will support stakeholders in identifying vulnerable and critical control points in order to limit the spread of contaminants.

Detailed knowledge on the complex connections in the European and worldwide spice trade network, gained from the surveys conducted within SPICED brought attention not only to the main sources/subjects of quality claims and the complexity of the spice trade network, but also to the points of vulnerabilities. Examples of vulnerable points included large distribution centres that are subject to strict quality control measures but their blockage or sabotage leads to major paralysation of the entire network, as well as small manufacturers or allotments including spice trade over the internet skipping effective quality control check. Network analysis among participants of the spice trade network revealed participants in roles of centrality and betweenness that facilitate further possible intervention into generating improved food safety within spice trade and distribution.

Using spice paprika production from field cultivation to packaged end-product as an example, points of vulnerability have also been described in the production technology including aspects related to both product composition and contamination. A finding in the survey on cultivation characteristics favourable for the technology and highly considered by the general public is that pesticide residues do not occur in paprika fruit and consequently in spice paprika upon authorised cultivation conditions, neither from the treatments nor from persisting environmental contamination. The survey also revealed that effective washing is an important element in the technology, capable to remove occasional pesticide residues, but of limited efficacy in the removal of microbial contaminants, unless the washing water is changed quite frequently. As main conclusion, the survey indicated that besides the quality of the raw material, drying and milling are the most sensitive production steps of the technology. Therefore, critical control points focus on these steps to provide absence of microbiological contamination and storability.

The consumption behaviour questionnaire resulted in detailed socio-demographic knowledge on spice preference and consumption in different countries and identified differences among various countries, drawing attention to the importance and need of public education in this field as knowledge of food safety aspects of herbs and spices were found unsatisfactory.

SPICED has also increased the knowledge on the possible contaminants, their behaviour in spice/herb matrices and during processing/production of these matrices, and the risk potential. For example, by performing tenacity studies on numerous agent–matrix combinations, the knowledge on biological agent survival in low moisture food like spices and dried herbs was brought a big step forward. The experiments on the biological agents of concern considered not only eight different spice and dried herb matrices, but also additional parameters. Among them were different spiking methods and/or storage temperatures. The results obtained on spiking techniques and tenacity data have been presented at various conferences and other events and will soon be published in peer-reviewed articles.

To control the safety of spices and herbs, proper methods for hazard detection are crucial. However, hazard detection and quantification can be a challenge in spices and herbs. These matrices often interfere with diagnostic methods due to inhibiting substances. Within SPICED, improving and establishing appropriate detection methods for the various targets and applications were major aims of the project. Substantial achievements were made including broad-range and high-throughput technologies. Besides new approaches, existing methods could be optimised for the detection of microbiological contaminants in spice and herb matrices. For example, optimised sample preparation using K_2SO_3 was successfully validated in a ring trial to facilitate the detection of *Salmonella* in the difficult spice matrix cinnamon. To detect also unknown chemical contaminants, non-targeted fingerprinting techniques in combination with chemometric modelling were established. Improved methodology will support the industry as well as official agencies regarding the control and monitoring the safety and authenticity in spices and herbs.

However, detection of hazardous contaminants also requires appropriate sampling. This is of particular importance since contaminations often occur very heterogeneously within a batch. Within the SPICED project, different sampling strategies were analysed and compared to identify the best approach.

SPICED further provides the scientific basis to improve monitoring and reporting systems, to ensure prevention and response on a high quality level. Existing systems were analysed and recommendations for improvement or standardisation were established. Early hazard detection will help to ensure the safety and authenticity of food that includes spices and herbs throughout the entire chain, and is essential to take corrective measures such as decontamination. To facilitate e.g. withdrawal, sufficient reporting of information necessary for food tracing is important. Rapid tracing of food is also crucial in case of an incident.

Effective decontamination of spices and herbs before usage as ingredients in processed food is of particular importance. However, decontamination is rather difficult in a medium with low water activity due to resistance of microorganisms, especially of those that have sporulated. The current decontamination technologies cover irradiation, steam treatment, and use of ethylene oxide. Although these techniques have been proven to significantly reduce microbial populations they either have some disadvantages on aroma and flavour or have poor consumer acceptance. Moreover, the use of ethylene oxide is prohibited in the EU due to its carcinogenic potential to humans. Therefore, the development of other potential decontamination techniques that could be used to produce high quality and microbiologically spices and herbs is of interest. Within SPICED the effect of different decontamination techniques on the microbial load, bioactive components and colour of spice paprika was compared, which results are of main importance for spice producers. However, the assessment of microbial decontamination methods of spice paprika also resulted in conclusions of substantial significance for the consumers, and therefore, to the general public. These indicate that i) steam treatment is accepted by the consumers and is technologically sufficient, but may influence product quality (content of certain bioactive compounds), and ii) irradiation is effective, yet it also affects product quality depending on irradiation dosage.

Data on the initial level of pathogens in spice and herb matrices, their tenacity during storage and their inactivation were combined within SPICED by applying predictive microbiology to relate them to a Food Safety Objective (FSO) and estimate public health effects. By using this approach it is possible to predict survival of food-borne pathogens in spices and herbs and when appropriate, to evaluate the control of their numbers and to reduce the risk of a food-borne disease outbreak or incident.

Overall, SPICED has strongly increased the knowledge on securing the spice and herb chains and protecting the consumer against potential biological and chemical contaminations of these products. Dissemination of the knowledge and expertise to the different stakeholder groups was ensured by numerous dissemination activities. Dissemination took place by all project partners on several occasions using various media covering digital materials and distribution thereof, personal presentations, posters, roll-ups, brochures as well as showpieces and haptic (and organoleptic) materials.

At the beginning of the project, several dissemination materials were established and designed in line with the project cooperate identity. The materials describe the background, major aim and objectives of the EU FP7 project and its structure, and present the SPICED consortium. The following materials were developed and updated as required:

- Project flyer in different languages

- Project compendia
- Poster
- Roll-up

In addition, an annual newsletter was published (with 3 issues) and further dissemination materials related to specific events were developed. Moreover, the SPICED website provided up-to-date information on the project and its activities. Further, all project partners promoted the SPICED project to interested stakeholders and the general public using also their institutional structures (e.g. institute webpage, annual reports). Information on the project was also published in other reports, press releases, and interviews and by a project video.

The project aims and outcomes have been presented and discussed at several public, governmental, and scientific events as well as during exhibitions, workshops and an international scientific symposium, which were organised by the SPICED consortium:

- So far, >150 oral and poster presentations were given by the SPICED project partners at various events addressing more than 20,000 stakeholders, from industry, government, media, the civilian and military sector. Events took place at national, EU, and global level.
- Dissemination of SPICED objectives/results, knowledge transfer, and expertise exchange also took place at several stakeholder events that were organised by the SPICED consortium. The international SPICED events covered four workshops (including one at the EXPO in Milan) and a symposium, with in total around 250 participants. Information on the SPICED Symposium “Spices and Herbs – A Risk Free Taste Experience?" including the book of abstracts and part of the presentations are available online: www.bfr.bund.de/en/event/spiced_symposium_spices_and_herbs___a_risk_free_taste_experience__-196629.html.
- In addition, a SPICED exhibition was created and presented at Science Centres in different European countries addressing particularly families.
- The SPICED project was further presented by the project partners to the general public and to interested stakeholders at additional events, for example at fairs and at the Long Night of Research in Austria.

Moreover, the results of the project have been and will be further communicated to the stakeholders in the form of publications. So far, several scientific and popular scientific articles and book chapters have been published. Further publications are planned/in progress including the publication of the results of the SPICED project in a special issue on SPICED. This special issue will be published by the peer-reviewed scientific journal Food Control and is foreseen to be available end of 2016/beginning of 2017. The tentative title of the special issue is: “Safety and Security of Spices and Herbs along Global Food Chains”. In addition, interviews, press releases, and other reports have been published that are accessible to the public.

SPICED has increased the awareness of the European spice and herb producers, the food safety authorities at the national, EU and international level and also of the consumers—since spices and herbs are small, but powerful. SPICED data will also stimulate discussions on whether more attention should be paid on neglected or under-researched issues for spreading possible contamination in the food chain—either by increased research activities, preventive actions or intensified information and training of the relevant stakeholders, including producers/processors, agencies as well as the consumers.

Next to improving the knowledge, the SPICED research activities have also identified further needs for future research, surveillance and additional risk prevention strategies within the spice and herb chains. To meet these challenges, the SPICED project has created a sustainable research network for future activities in the field of food safety on some of the most diverse food chains.

Gender equality measures were applied within the whole SPICED project and the number of female employees, including the management, has increased within the project, overcoming the 50% barrier for the major part of the project duration.

Project public website address and project consortium

Further information on the project consortium and the project's background, objectives, workpackages, and activities (including the project newsletters and a video) are available on the project website: www.spiced.eu

The SPICED consortium was composed of eleven project partners from industry, academia and food authorities in seven EU countries and four integrated stakeholders as listed in the following:

Project partners

- Bundesinstitut für Risikobewertung (Federal Institute for Risk Assessment), Germany (coordinator)
- Austrian Agency for Health and Food Safety, Austria
- Institute of Food Safety, Animal Health and Environment, Latvia
- DLO Foundation – RIKILT, the Netherlands
- FUCHS Gewürze GmbH, Germany
- National Agricultural Research and Innovation Centre, Hungary
- RTD Services, Austria
- University of Limerick, Ireland
- National Agricultural and Food Centre, Slovakia
- Bundeswehr Research Institute for Protective Technologies and NBC-Protection, Germany
- Wageningen University – Laboratory of Food Microbiology (FHM), the Netherlands

Integrated stakeholders

- European Spice Association, Germany
- Fachverband der Gewürzindustrie, Germany
- Van Hees GmbH, Germany
- Kräuter Mix GmbH, Germany