SPICED
spices & herbs

**ABSTRACTS**
SPICED SYMPOSIUM,
1ST – 2ND JUNE 2016

BfR
Bundesinstitut für Risikobewertung

# SPICES & HERBS – A Risk-Free Taste Experience?

In cooperation with

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# SPICED Symposium
# Spices and Herbs –
# A Risk-Free Taste Experience?

1–2 June 2016, Berlin, Germany

## Imprint

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Spices and Herbs – A Risk-Free Taste Experience?

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Dear participants,

We welcome you to the international SPICED Symposium “Spices and Herbs – A Risk-Free Taste Experience?” at the Federal Institute for Risk Assessment (BfR) in Berlin.

The unique 2-day symposium spotlights the minor food ingredients spices and culinary herbs in the view of food safety. The symposium was initiated by the European Union (EU) funded project SPICED, which aims at securing the spices and herbs commodity chains in Europe against deliberate, accidental or natural biological and chemical contamination (www.spiced.eu).

The EU market is one of the largest markets for spices and culinary herbs in the world. Mostly, these commodities are imported as dried raw materials from producing regions outside of the EU. Meeting the demands of the EU can be a challenge for the involved stakeholders. Although being minor food ingredients, spices and herbs can contain potential hazardous contaminants. Contaminations can take place at numerous vulnerable points within production and supply chains and can pose a serious risk for consumers.

Contaminations of spices and herbs with biological and chemical agents may occur for example naturally or accidentally. Microorganisms can be easily introduced during primary production, processing, storage, and transport and some microorganisms are able to stay viable even in dried culinary herbs and spices. Microbial contaminated spices and herbs can serve as a carrier to introduce microorganisms into other foodstuffs. Depending on the properties of such foodstuffs, failure in hygiene measures might lead to microbial proliferation and toxin production and can finally cause a food-borne illness. Typical natural/accidental chemical contaminants of spices and herbs cover e.g. mycotoxins, heavy metals, persistent organic pollutants, and in a broader sense also pesticide residues. Even in minor food ingredients, such chemical hazards might be present at levels that can represent a health risk.

In addition, these valuable commodities can be deliberately adulterated because of possible economic benefits. Food fraud causes not only economic losses to other stakeholders, but also raises health concerns. For example, addition of Sudan dyes is an illegal and harmful way to improve the visual quality of spices such as paprika/chilli, curcuma, and curry. These azo dyes have the potential to give rise to carcinogenic aromatic amines.

The identification of contaminated spices and herbs as a cause of a food-borne infection or of an intoxication would be difficult, because consumers and experts often focus on major food ingredients. Moreover, many detection methods are less suitable for the heterogeneous herb/spice matrices. Therefore, the symposium sets a special focus on the challenges in establishing and maintaining food safety in the spice and herb chains and covers sessions on: I) Spice and herb chains, II) Microbiological hazards in spices and herbs, III) Food fraud and chemical hazards in spices and herbs, and IV) Decontamination methods and food control.

We are pleased to welcome stakeholders from industry, government, and academia to the SPICED Symposium and wish you two stimulating days with oral and poster presentations, fruitful discussions, and an inspiring exchange with all participants.

Dr. Juliane Bräunig (Coordinator of SPICED), the SPICED Consortium, and
Professor Dr. Dr. Andreas Hensel

# 1 Programme

## Wednesday, 1 June 2016

### Opening ceremony

12:30–12:40 p.m.
**Welcome**
Reiner Wittkowski, Federal Institute for Risk Assessment (BfR), Berlin, Germany

12:40–12:55 p.m.
**Welcome and introduction to food safety**
Michael Winter, Federal Ministry of Food and Agriculture (BMEL), Bonn, Germany

12:55–1:10 p.m.
**Introduction to the symposium and the SPICED project**
Juliane Bräunig, Federal Institute for Risk Assessment (BfR), Berlin, Germany

### Session I: Spice and herb chains

*Chairs:* András Székács, National Agricultural Research and Innovation Centre (NARIC), Budapest, Hungary; Dirk Radermacher, German Spice Association, Bonn, Germany

1:10–1:40 p.m.
***Keynote***
**Challenges in the production of safe spices and herbs**
Gerhard Weber, European Spice Association (ESA), Bonn, Germany

1:40–2:00 p.m.
**Network and vulnerability analyses of international spice trade**
Zoltán Lakner, Budapest Corvinus University, Budapest, Hungary

2:00–2:15 p.m.
**Vulnerable points in spice production chains**
Nóra Adányi, National Agricultural Research and Innovation Centre (NARIC), Budapest, Hungary

2:15–2:30 p.m.
**Protocol of sampling technologies and corresponding statistics**
Jennifer Banach, DLO Foundation – RIKILT (DLO), Wageningen, The Netherlands

*2:30–3:00 p.m. Coffee break*

**Wednesday, 1 June 2016**

**Session II: Microbiological hazards in spices and herbs**
*Chairs:* Anneluise Mader, Federal Institute for Risk Assessment (BfR), Berlin, Germany; Aivars Bērziņš, Institute of Food Safety, Animal Health and Environment (BIOR), Riga, Latvia

3:00–3:30 p.m.
***Keynote***
**Overview on biological hazards and detection methods**
Anselm Lehmacher, Institute for Hygiene and Environment, Hamburg, Germany

3:30–3:40 p.m.
**Biological hazards and their tenacity in spices and dried herbs**
Anneluise Mader, Federal Institute for Risk Assessment (BfR), Berlin, Germany

3:40–4:00 p.m.
**Detection and tenacity of *Salmonella***
Philipp Lins, Austrian Agency for Health and Food Safety (AGES), Innsbruck, Austria

4:00–4:15 p.m.
**Survival, detection and toxinogenic potential of *Bacillus cereus* group species in spices and herbs**
Hendrik Frentzel, Federal Institute for Risk Assessment (BfR), Berlin, Germany

4:15–4:30 p.m.
**Detection of *Staphylococcus aureus* and *Listeria monocytogenes* DNA in artificially contaminated samples**
Svetlana Cvetkova, Institute of Food Safety, Animal Health and Environment (BIOR), Riga, Latvia

4:30–4:45 p.m.
**Impact of spiking techniques on the survival of *Staphylococcus aureus* in artificially contaminated condiments**
Mai Dinh-Thanh, Federal Institute for Risk Assessment (BfR), Berlin, Germany

*4:45–5:15 p.m. Break*

5:15–5:35 p.m.
**Predictive microbiology**
Marcel Zwietering, Wageningen University (WU), Wageningen, The Netherlands

5:35–5:45 p.m.
**Predictive microbiology for spices and herbs**
Ioanna Stratakou, Wageningen University (WU), Wageningen, The Netherlands

5:45–6:00 p.m.
**Available community tools for predictive modelling**
Matthias Filter, Federal Institute for Risk Assessment (BfR), Berlin, Germany

6:00–6:30 p.m.
**Poster session**

*6:30 p.m. Dinner (at the venue)*

**Thursday, 2 June 2016**

**Session III: Food fraud and chemical hazards in spices and herbs**
*Chairs:* Saskia van Ruth, DLO Foundation – RIKILT (DLO), Wageningen, The Netherlands; Carsten Fauhl-Hassek, Federal Institute for Risk Assessment (BfR), Berlin, Germany

9:00–9:30 a.m.
***Keynote***
**Quality assurance of spices and herbs in official control**
Sandra Schumacher, Chemical and Veterinary Investigations Office (CVUA), Karlsruhe, Germany

9:30–9:45 a.m.
**Detection of natural and accidental contamination of spices and herbs**
Vadims Bartkevičs, Institute of Food Safety, Animal Health and Environment (BIOR), Riga, Latvia

9:45–10:15 a.m.
***Keynote***
**A proposed comprehensive strategy to detect the fraudulent adulteration of herbs: The oregano approach**
Christopher Elliott, Queen's University Belfast, United Kingdom

10:15–10:30 a.m.
**Deliberate contamination – Detection by spectrometric fingerprinting methods**
Saskia van Ruth/Isabelle Silvis, DLO Foundation – RIKILT (DLO), Wageningen, The Netherlands

10:30–10:45 a.m.
**Deliberate contamination – Detection by spectroscopic fingerprinting methods**
Bettina Horn, Federal Institute for Risk Assessment (BfR), Berlin, Germany

*10:45–11:15 Coffee break*

**Session IV: Decontamination methods and food control**
*Chairs:* Bernd Appel, independent scientific expert working for the Federal Institute for Risk Assessment (BfR), Berlin, Germany; András Székács, National Agricultural Research and Innovation Centre (NARIC), Budapest, Hungary

11:15–11:45 a.m.
***Keynote***
**New approaches for the decontamination of spices and herbs**
Oliver Schlüter, Leibniz Institute for Agricultural Engineering Potsdam-Bornim (ATB), Potsdam, Germany

11:45 a.m.–12:00 p.m.
**Decontamination of spice paprika**
Ildikó Bata-Vidács, National Agricultural Research and Innovation Centre (NARIC), Budapest, Hungary

12:00–1:30 p.m.
*Lunch and* **Poster session**

**Thursday, 2 June 2016**

**Session IV: Decontamination methods and food control (continued)**

1:30–2:00 p.m.
***Keynote***
**Import control of food of non-animal origin**
Ute Gramm, Authority for Health and Consumer Protection, Hamburg, Germany

2:00–2:20 p.m.
**Overview on the Rapid Alert System for Food and Feed (RASFF)**
David Trigo, Federal Office of Consumer Protection and Food Safety (BVL), Berlin, Germany

2:20–2:35 p.m.
**Forward-backward tracing with FoodChain-Lab: Software supporting foodborne disease outbreak investigations**
Armin Weiser, Federal Institute for Risk Assessment (BfR), Berlin, Germany

2:35–2:50 p.m.
**Recommendations for an effective monitoring system for the most relevant hazards in the spices and herbs chain**
Jennifer Banach, DLO Foundation – RIKILT (DLO), Wageningen, The Netherlands

2:50–3:00 p.m.
**Farewell**
Juliane Bräunig, Federal Institute for Risk Assessment (BfR), Berlin, Germany

# 2 Abstracts

## 2.1 Welcome and introduction to food safety

Michael Winter

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The provision of safe, nutritious, high quality and affordable food to Europe's consumers is the central objective of EU and Germany´s policy, which covers all stages of the food supply chain, “from farm to fork”. Its standards and requirements aim to ensure a high level of food safety and nutrition within an efficient, competitive, sustainable and innovative global market. Every farmer, food manufacturer, baker or butcher has to ensure, that its products are safe by performing company´s own checks. The competent food safety authorities perform official controls for the verification of compliance with food and feed law, animal health and animal welfare rules through risk-oriented company checks and official sampling.

Imported food products, such as exotic spices have to comply with the same food safety requirements as food products which are produced in the EU. However globalised and complex distribution channels are an important challenge to the local food safety authorities. If possible, risk-oriented samples are to be taken before the products are on the market. In cases of serious non-compliances suspension of imports of the food or feed in question or reinforced checks can be decided on EU or national level.

Food fraud poses increasingly a challenge for the enforcement along the food chain. High profit margins and the low risk of being prosecuted have led to misrepresentation and deception becoming widespread. Food fraud damages the industry and may endanger consumer health. Therefore both the EU and Germany put in place series of measures to fight food fraud. These include, *inter alia*, the revision of the EU Regulation 882/2004 on official controls; to strengthen the fight against fraud and to improve information-sharing among Member States on fraud. The EU has dedicated a network of administrative assistance for Food Fraud Contact Points in the Member States that handle specific requests for cross-border cooperation in cases of “food fraud”. It is also foreseen to establish a European Union reference centre for the authenticity and integrity of the food chain. Within Germany also federal contact points for food fraud were appointed and a national program to fight food fraud is developed by the Federal Office of consumer protection and food safety (BVL). The national program includes a national expert advisory body on food fraud as well as an early warning system. The BMEL also financially supports research in the field of novel methodology for the authentication and traceability of food and feed, including on spices and herbs.

## 2.2 Introduction to the SPICED project

Juliane Bräunig[1] and the SPICED Consortium

[1] Federal Institute for Risk Assessment (BfR), Department Biological Safety, Berlin, Germany
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The EU project SPICED (www.spiced.eu), which started in July 2013, aims at securing the spices and herbs commodity chains in Europe against deliberate, accidental or natural biological and chemical contamination. The project consortium is composed of eleven experienced institutions from seven European countries (Austria, Germany, Hungary, Ireland, Latvia, The Netherlands, and Slovakia). These project partners are supported by integrated stakeholders with significant knowledge of the worldwide spices and herbs markets including all levels of production and sale. The unique consortium includes partners from industry, academia, and food authorities and represents a balanced network of experts comprising different areas along the spice/herb supply chains.

The SPICED research work is performed within four work packages: Matrix Chains and Modelling; Biological Hazards; Chemical Hazards; and Prevention and Response. Within these work packages, information has been systematically collected and evaluated e.g. on spice/herb producing operations and on potential hazardous contaminants to identify vulnerable points and the most important hazards and to assess the survival capacities of microbiological agents. Artificial spiking experiments using different inoculation approaches further improved the knowledge on the properties of biological hazards in spices and dried herbs. Reliable detection methods for biological hazards in spices and herbs have been optimised and new methods, including high-throughput methods, have been established. To reduce natural and accidental as well as deliberate contaminations of spices and herbs with (unforeseen) chemical agents and to ensure authenticity of spices and herbs, non-targeted fingerprinting methods have been developed. Moreover, to improve prevention and response to chemical adulterations and foodborne incidents caused by contaminated spices and herbs, the possibilities and limitations of available and improved alerting, reporting, and decontamination systems have been evaluated for the spice/herb chains.

Part of the outcome of the SPICED project that will end in June 2016 will be presented at the current symposium.

Acknowledgement: The SPICED project received financial support from the 7th Framework Programme of the EU (Grant Agreement No. 312631).

## 2.3 Challenges in the production of safe spices and herbs

Gerhard Weber

ESA Secretary General, European Spice Association (ESA)
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Approx. 350 spice companies located in the EU are marketing spices in Europe. Associations and companies from Non-EU countries (countries of origin) are associate members of the European Spice Association (ESA) to embrace the supply chain assuring the transfer of knowledge about legislation, quality, hygiene into the origins and vice versa.

The spice industry produces spices, herbs, blends thereof, seasonings, condiments and technical blends for artisanry (e.g. baker, butcher), industry, catering, private households.

The spice industry

- buys raw material (e.g. dried herbs and spices)
- selects suppliers
- imports, tests
- cleans, standardises, packages and
- puts spice products on the market.

Quality issues of herbs and spices:

- Pesticide residues
- Nicotine in mushrooms (e.g. boletus), herbs and spices
- Biphenyl in herbs and spices, especially nutmeg and mace
- Dioxins in paprika powder, dried basil, marjoram
- Traces of DEET in some spices
- Aflatoxins, ochratoxins,
- Chlorate, perchlorate,
- Anthraquinone, polycyclic aromatic hydrocarbons (PAH),
- Mercury,
- Pyrrolizidine alkaloids,
- Mineral oil compounds (MOSH/MOAH),
- Unintended presence of traces of allergens.

The spice industry has developed sophisticated systems to meet the requirements of food legislation, customer and consumer demands.

Technical Commissions are established by the national associations (in the EU) to discuss current and emerging issues <u>at national level</u>. National experts are working in the ESA Technical Commission (TC) dealing with current and emerging issues <u>at EU level</u>; working groups take care of specific issues and report to the TC.

Specific subjects are dealt with in close cooperation with the appropriate partners, e.g. ASTA, VPA, ISB.

In the spice sector problems can arise from pesticide application, drying processes, storage/transportation, climate/weather conditions, volcano activity, fire, political instability, …

Networking and communication are key.
The spice business is global, so are the challenges, the cooperation and the spirit.

## 2.4 Network and vulnerability analysis of international spice trade

Zoltán Lakner[1], Erzsébet Szabó[2], Viktória Szűcs[2], András Székács[2]

[1] Budapest Corvinus University, Budapest, Hungary
[2] National Agricultural Research and Innovation Centre, Budapest, Hungary

**Background**
Long-distance trade of spices had formed one of the first global product trade networks. The spice trade developed through South Asia and Middle East in around 2000 BC with cinnamon and pepper. In the book of Genesis of the Old Testament, holly text of Judaeo-Christian culture, Joseph was sold into slavery by his brothers to spice merchants.

Since the 1960s, global spice trade has been increasing at an exponential rate, faster than food production itself. Spice fluxes between countries form a complex, dynamic web of interactions. Spice trade has numerous specific aspects compared to trade of the majority of agro-food products, because (1) there is a considerable geographic distance between the most important suppliers and buyers. That's why the international spice trade embraces a considerable, long-distance transport and difficult logistical system. (2) The most important spice producing countries are the relatively lesser developed ones: India, Bangladesh, Pakistan, Iran, Nepal, Colombia, Ethiopia and Sri Lanka are among the top ten most important exporters. Increasing political instability in a number of important middle and Central Asian countries have further increased the unpredictability of some key actors (nodes) in spice chain. (3) Spices are widely used in a range of products (e.g. in canning, poultry processing and meat industry) as well as in gastronomy. Under these conditions, an eventual food safety problem at spices can cause a considerable economic and long-range moral loss.

However, the importance of spice trade is widely acknowledged, the structure, conduct and performance of this network is hardly known. The aim of research has been to analyse and model the international spice trade network, based on long-range data. Novelties of research are: (1) application of network science concept and methodology on analysis of international spice flows; (2) determination of key players and dynamics of spice trade network; (3) determination of efficiency of food safety control mechanism of EU, based on comparative analysis of trade and food safety network data; (4) modelling of vulnerability of spice trade network by agent-based simulation.

**Methods**
Based on Eurostat we have prepared a database on international trade flow of red pepper (paprika) and black pepper within the member states of the EU. On this base we have been able to determine the characteristics of flow within the member states of products, containing red or black pepper. This flow has been modelled and analysed by network-simulation methods.

**Findings**
A frequently used measure in the structural analysis of complex networks is the node- or edge-betweenness centrality. It quantifies how "central" is the position of the node/edge in the network, in the sense that high centrality nodes/edges collect large portions of the traffic through the network. For this reason, they also present the Achilles' heel of a network as changes in the status of these nodes and edges will have the largest effect on the whole system, both in connectivity and transport properties. Nodes with top centrality values play a critical role in the spice trade network, because any food-born substance (e.g. chemical or microbiological contamination) will spread most efficiently through them into the rest of the network, while tracing the source of such a substance is difficult due to the large number of network paths running through these nodes. Fast spread is also facilitated by the small value

of the average shortest path (measured in hop-counts) of the network. By methods of simulation we have been able to determine the basic patterns of spice trade and identify the most vulnerable points.

**Implications**
Based on this approach we are able to determine the most critical vertices and edges in European spice trade networks. On this base we are able to develop a risk-based control strategy as well as in case of any accidental or purposeful spice related food safety problem there is a possibility to develop and optimise risk-management plans.

## 2.5 Vulnerable points in spice production chains

Nóra Adányi[1], Helga Molnár[1], Ildikó Bata-Vidács[2], Mária Mörtl[2], Rita Tömösközi-Farkas[1], András Székács[2]

[1] Food Science Research Institute, NARIC, Hungary-1022 Budapest, Herman Ottó u. 15.
[2] Agro-Environmental Research Institute, NARIC, Hungary-1022 Budapest, Herman Ottó u. 15.

Spice paprika is a major spice commodity in the European Union (EU), produced locally and imported from non-EU countries, reported not only for chemical and microbiological contamination, but also for fraud. To demonstrate the importance of various contamination factors in the Hungarian production and EU trade of spice paprika, several aspects concerning food safety of this commodity will be presented. Quality assurance measures established along the spice paprika production technology are surveyed with main critical control points (CCPs) identified. External factors, such as production factors, harvest procedures, storage duration and temperature, processing protocols, conditions exacerbating water loss or post-ripening affect the quality of the commodity. Drying and milling are the most critical steps of spice paprika processing. Quality, nutritive value, and storage stability depends, to a high extent, on the conditions at which drying and milling are performed. Accordingly, in the production technology, there are three CCPs in the production line: at the drying step, at the microbial decontamination stage, and at the compositional and contamination checking of imported half-products.

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## 2.6 Protocol of sampling technologies and corresponding statistics

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**Introduction**: Because of the variability in both the strategy for collecting samples and sample test results, some contaminated lots may incidentally be classified as acceptable. The choice of the sampling strategy is very important in the development of any sampling plan.

**Purpose**: This study aims to develop an optimisation model to determine cost-effective sampling plan for detecting chemical hazards over the control points along the spices and herbs supply chain.

**Methods**: A simulation model was developed to compare different sampling strategies (simple random sampling (SRS), stratified random sampling, (STRS) and systematic sampling (SS)) with respect to their ability to detect a chemical or microbial contamination in a spices or herbs lot. In addition, an optimisation model was developed to determine cost-effective sampling plan for detecting chemical hazards over the control points along the spices and herbs supply chain using mixed integer linear programming (MILP) method. Based on the results, recommendations for sampling were provided to the industrial partner (FUCHS).

**Results**: The simulation results showed that in lots with low levels of contamination (1 % and 2 %), the SS strategy is preferred and the number of samples to be taken should be 100 %. At the higher contamination rates (5 % and 10 %), the STRS strategy is preferred and the number of samples to be taken should be higher or equal to 50 %.

**Significance:** This model can aid governmental authorities and industry in the evaluation of the applied sampling strategies and the development of an optimal sampling protocol of spices and herbs.

Acknowledgements: This research was executed in the framework of the EU project SPICED (Grant Agreement: 312631) with the financial support from the 7th Framework Programme of the EU. Financial contributions from the Dutch Ministry of Economic Affairs are acknowledged. We kindly thank Prof. Marcel Zwietering and Ioanna Stratakou (FHM, Wageningen University) for their valuable input to this study.

## 2.7 Overview on biological hazards and detection methods

Anselm Lehmacher

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Spices and dried herbs were implicated in foodborne diseases and outbreaks. Most of them were caused by *Salmonella*, followed, in descending order, by *Bacillus cereus*, *Clostridium perfringens*, and *C. botulinum*. However, only low initial numbers of pathogens are likely to be present in untreated herbs and spices. Therefore, several outbreaks with low attack rates were linked to low infective doses of *Salmonella*e from contaminated spices and dried herbs. In some of these outbreaks, especially infants and elderly people were infected. In particular strains of *Salmonella* serovars, rarely isolated from patients, caused these outbreaks.

In many spices and herbs, mesophilic bacilli predominate among the microbial flora. Thus, *Bacillus cereus* is frequently detected in spices and dried herbs. But high counts up to $10^5$ CFU/g, a prerequisite of a toxi-infection with enterotoxigenic *B. cereus*, have only exceptionally been determined. The vast majority of *B. cereus* isolates encode at least one enterotoxin. A few outbreaks traced to *B. cereus*-contaminated spices have been reported in recent years.

In several spices, anaerobic *Clostridium perfringens* has also been found with a relatively high incidence. Usually its number did not exceed 1000 CFU/g. Outbreaks traced to spices contaminated with *C. perfringens* were reported from Denmark in 2011. In recent decades, neurotoxigenic *Clostridium botulinum* caused a few outbreaks related to garlic in oil, mustard, prepared with fried lotus rhizomes, and chamomile tea.

Although *Salmonella*, *Bacillus cereus*, *Clostridium perfringens*, and *C. botulinum* are unable to multiply in dried foods, they are well adapted to this environment with low moisture. They survive up to years in dried foods. Toxigenic bacilli and clostridia form spores resistant to cooking temperature and desiccation. *Salmonella* cells are injured, resting or might enter a viable-but-not-culturable state during their survival in low moisture. Therefore, classical detection methods might underestimate the numbers of *Salmonella*e. The resuscitation and the overcoming of the bactericidal activity of spices and herbs are of particular concern in the detection of bacterial pathogens, especially when herbs and spices are consumed raw or added to prepared foods without further cooking. Cleaning, curing, drying, grinding, and pulverising of herbs and spices have no or little effect on the initial microbial flora. Adapted pathogenic bacteria will multiply in food of higher moisture once the contaminated spice is added.

In conclusion, herbs and spices are infrequent contributors to foodborne diseases and outbreaks. But low numbers of well adapted pathogenic bacteria are probably to be present in spices and dried herbs.

## 2.8 Biological hazards and their tenacity in spices and dried herbs

Anneluise Mader[1] and the SPICED Consortium[2]

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Spices and herbs might be present in almost every processed food and are hence reaching a spaciously distribution although they are only minor compounds. 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 in particular obvious as spices have been the source of various foodborne outbreaks. At least 22 outbreaks were 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.

Qualitative and quantitative detection methods including sample preparation methods for various biological-hazards/spices-and-herbs-matrix-combinations were tested and optimised by the SPICED project consortium. It could be shown that the detection methods available are limited for several combinations as various inhibitory factors can influence the analyses.

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 have been tested also taking possible natural contamination pathways into account. Beside significant differences in the survival time between vegetative cells and endospores, it could be shown that the spiking technique as well as the spice and herb matrices themselves can have a significant impact.

Acknowledgement: This research was executed in the framework of the EU project SPICED (Grant Agreement: 312631) with the financial support from the 7th Framework Programme of the EU. This publication reflects the views only of the authors, and the European Commission cannot be held responsible for any use which may be made of the information contained therein.

## 2.9 Detection and tenacity of *Salmonella*

Philipp Lins

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Dried spices and herbs represent small ingredients with a water activity of < 0.6, which is considered below the minimum for microbial growth. Nevertheless, they pose a significant health risk because condiments are often added to a dish directly before consumption or used for ready-to-eat foods with no further germ reduction step. The European Food Safety Authority (EFSA) and the Rapid Alert System for Food and Feed (RASFF) place *Salmonella* spp. in spices and herbs among the most relevant agent-matrix combinations. *Salmonella* spp. are pathogens with a very low infectious dose of ≤ 1–10 colony forming units (CFU) per gram of a product, and thus are not allowed to be detected in 25 g of a product.

Nine spices and herbs were checked for their initial microbial status, moisture content and water activity, and different spiking strategies for *Salmonella* Oranienburg (originally isolated from ground cumin) were evaluated. Furthermore, antimicrobial effects against *S*. Oranienburg were determined either with short-term experiments with 1:10 (w/w) dilutions of the matrices or dry spiking, simulating common household storage conditions. Additionally, the ISO 6579 for the detection of *Salmonella* spp. was modified (mISO; modified ISO) by the supplementation of $K_2SO_3$ to the buffered peptone water (BPW) as well as an increase of the initial dilution to 1:20 (w/w) prior enrichment. Subsequently, the mISO was compared with the loop-mediated isothermal amplification via the Molecular Detection System (MDS) from the company 3M towards the detection of 14 *Salmonella* serovars/isolates. Moreover, the limit of detection (LOD) for *S*. Oranienburg of the mISO and the MDS was determined within BPW, and was compared within cinnamon and oregano, matrices proven to be highly antimicrobial active. Last but not least, a ring trial was run among the partners of the EU project SPICED with regard to detect *Salmonella* spp. in 25 g of cinnamon.

Based on the results, several significant antimicrobial effects were determined, emphasising the importance of the initial non-selective enrichment to improve the LOD for *Salmonella* detection. After an appropriate enrichment, the LOD for the mISO and the MDS were comparable with < 5 CFU per 25 g of a matrix, except for oregano, where further improvements are required. The MDS has been proven to be a reliable high-throughput tool where 96 samples are analysed within 75 min, pointing to a potential reduction of the analysis time, especially as a negative-screening.

Acknowledgement: This research was executed in the framework of the EU project SPICED (Grant Agreement: 312631) with the financial support from the 7th Framework Programme of the EU.

## 2.10 Survival, detection and toxinogenic potential of *Bacillus cereus* group species in spices and herbs

Hendrik Frentzel, Mai Dinh Thanh, Gladys Krause, Bernd Appel, Anneluise Mader

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*Bacillus* (*B.*) spp. are Gram-positive spore forming bacteria that are often present in dried herbs and spices. Several foodborne disease outbreaks caused by *Bacillus* spp., particularly *B. cereus*, could be associated with contaminated spices (EFSA 2013). *B. cereus* is able to produce up to three different enterotoxins causing diarrhoea (non-haemolytic enterotoxin, haemolysin BL and cytotoxin K) and/or the emetic toxin cereulide. The ability for toxin production can differ between the members of the *B. cereus* group (also called *B. cereus* sensu lato) that comprise the following species: *B. cereus* (sensu stricto), *B. anthracis*, *B. weihenstephanensis*, *B. thuringiensis*, *B. mycoides*, *B. pseudomycoides* and *B. cytotoxicus*. However, standard ISO methods for the detection of *B. cereus* are not suitable to discriminate between these species.

Within the SPICED project we wanted to answer the following questions:

(1) Which *B. cereus* group species can be found in spices and herbs and which toxin genes do they carry? (2) How long do *B. cereus* spores remain viable in spices and herbs? (3) Is it possible to detect individual *B. cereus* group species in spices and herbs by PCR without prior cultivation?

The occurrence of *B. cereus* (sensu lato) was investigated in dried and ground allspice, basil, cinnamon, nutmeg, oregano, paprika, parsley and pepper in accordance with ISO 7932. Species identification and detection of toxin genes from isolates were conducted by multiplex real-time PCR. We detected *B. cereus* (sensu lato) in allspice, basil, oregano, paprika, parsley and pepper in a range of 10 to 1000 CFU/g. Of 59 isolates, 52 were identified as *B. cereus* (sensu stricto), four as *B. weihenstephanensis*, two as *B. mycoides* and one as *B. thuringiensis*. Except for the *B. mycoides* strains, all isolates carried at least two toxin genes.

The survival of *B. cereus* spores was investigated by mixing 0.5 g sand inoculated with spore suspension (dried overnight) with 4.5 g spice matrix to reach a final concentration of approximately $10^5$ CFU/g. The samples were stored at 23 ± 1 °C in the dark. For all matrices, only a slight reduction of less than one log level CFU/g could be observed within 50 weeks.

For the detection and discrimination of four individual *B. cereus* group species in condiments, we used multiplex real-time PCR after testing different DNA extraction methods. The five tested commercially available DNA extraction kits were not suitable for the extraction of bacterial spore DNA from spices and herbs. In contrast, using an extraction method based on DNA precipitation with cetyltrimethylammonium bromide (CTAB) and removal of inhibitory substances by chloroform we could detect *B. cereus* spores in spiked oregano, paprika and pepper, but not in spiked allspice.

Our results show that spices and herbs may contain members of the *B. cereus* group with the potential to produce toxins. However, for toxin production the condiments need to be added to foods with higher water content, which are stored under improper temperature conditions. *B. cereus* spores in condiments remain stable and germinable for very long time. For the detection of individual *B. cereus* group species the CTAB method combined with real-time PCR is an alternative to cultural standard methods.

Acknowledgement: This work was performed within the EU project SPICED (Grant Agreement: 312631; www.spiced.eu) with financial support from the 7th Framework Programme of the EU.

## 2.11 Detection of *Staphylococcus aureus* and *Listeria monocytogenes* DNA in artificially contaminated samples

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Direct culture-independent detection of bacterial pathogens based on molecular methods provides an excellent time-saving tool for identification and characterisation of microorganisms found in food.

DNA isolation from spices and herbs using CTAB (cetyltrimethylammonium bromide) method with subsequent bacterial DNA detection by PCR was carried out on two microorganisms: *Staphylococcus aureus* and *Listeria monocytogenes*.

Total DNA was extracted from spices and herbs artificially contaminated with bacterial suspensions containing different concentrations ($10^6$ to $10^2$ CFU/ml) of *S. aureus* or *L. monocytogenes*.

Two spices (cinnamon and vanilla) and two herbs (parsley and basil) were used in experiments with *S. aureus*. A conventional PCR targeting *S. aureus* specific 16S rRNA gene successfully detected $10^2$ CFU/g in basil, parsley and vanilla. Limit of detection (LOD) of $10^4$ CFU/g was determined for cinnamon.

Along with “classical” CTAB isolation of DNA a CTAB protocol with addition of polyvinylpyrrolidone (PVP) was tested on spices (black pepper, paprika/chili, cinnamon, nutmeg, allspice) and herbs (basil, parsley, oregano) artificially contaminated with *L. monocytogenes*.

Combination of CTAB+PVP DNA isolation protocol with SYBR Green real-time PCR targeting *L. monocytogenes prfA* gene had LOD of $10^3$ CFU/g for basil and paprika/chili. LOD of $10^4$ CFU/g was obtained for black pepper, oregano and parsley. LOD of $10^5$ CFU/g was determined for nutmeg and only level of contamination $10^6$ CFU/g gave positive signal in allspice and cinnamon.

Use of CTAB method for direct DNA isolation was successful for all spices and herbs included in this study. Additional research might be needed for difficult matrices like nutmeg, allspice and cinnamon to improve DNA recovery and PCR performance.

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## 2.12 Impact of spiking techniques on the survival of *Staphylococcus aureus* in artificially contaminated condiments

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**Background**: Culinary herbs and spices may be contaminated with pathogenic microorganisms such as *Salmonella* spp., *Bacillus cereus*, and *Staphylococcus* (*S.*) *aureus* (McKee 1995, Sospedra *et al*. 2010). Since condiments are added to various foods, they can act as vehicle of pathogenic bacteria. Therefore, understanding of the survival properties of pathogenic microorganisms in condiments is of utmost importance for consumer protection. An approach to investigate the survival capacities are tenacity studies, which often require an artificial contamination (spiking) step. Likewise, natural contamination of condiments may take place at different steps along the whole production chain, *i.e.* during cultivation as well as at harvest, drying, processing, and storage. Therefore, several spiking techniques might be needed to mimic the various contamination pathways. The objective of our study was to investigate the effect of the spiking technique on the survival of *S. aureus* in condiments.

**Methods**: Condiments (dried and ground paprika, pepper, and oregano) were inoculated with *S. aureus* using four different spiking techniques: (1) an aqueous culture was air-dried directly on the condiment; (2) an aqueous culture was air-dried on sand, then added to the condiment; (3) an aqueous culture or (4) a culture in nutrient media with lyoprotectant was freeze-dried and then crushed to powder, followed by adding condiment into the vial. Bacterial counting was conducted according to ISO 6888-1. All samples were stored in the dark and tested in triplicates. The survival rate of *S. aureus* in the different condiments over 25 weeks was fitted to mathematical survival models and D values were calculated.

**Results**: Results indicated that, desiccation reduced the *S. aureus* initial count after spiking. Survival of *S. aureus* after drying was dependent on the spiking technique and varied between the three different condiments. The calculated D values at 23 °C, which ranged from 1.4±0.07 days (paprika, technique 3) to 45.92±8.65 days (oregano, technique 4) emphasised again that the spiking technique and the matrix itself affected the stability and survival of *S. aureus* in herbs and spices during 25 weeks storage.

**Conclusion**: Stability and survival of *S. aureus* in artificially contaminated condiments depend on the spiking technique used. There is no technique that suits to every investigation purpose. Therefore, at least two different spiking techniques should be considered for tenacity studies. Considering a stable starting inoculum, techniques (2) and (4) performed best in our study.

Acknowledgement: This work was performed within the EU project SPICED (Grant Agreement: 312631; www.spiced.eu) with financial support from the 7th Framework Programme of the EU.

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## 2.13 Predictive microbiology

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Modelling in quantitative food microbiology makes use of mathematical equations to describe biological processes. These models can be used to perform various tasks like quantifying phenomena, testing significant differences, investigate correlations, investigate mechanisms, designing experiments, determine the performance of sampling plans, or describing kinetics within a food chain to design control of food safety. It is important to clearly identify what one wants to realize, and depending on the purpose of the model, select the most appropriate approach for modelling. Various applications of modelling are illustrated and for each application an example is given to highlight the broad use of models and model results in food microbiology. Specifically the use of models in quantitative risk assessment and sampling plans will be highlighted.

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## 2.14 Predictive microbiology for spices and herbs

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Spices and herbs can be contaminated with high numbers of microorganisms among them pathogens and therefore it is important to estimate the risk of consumption of these commodities. A concrete approach to estimate such risk is the Food Safety Objective (FSO) concept linked to the appropriate level of protection (ALOP). The FSO integrates factors such as the initial numbers, the sum of reductions and the sum of increases. These FSO components and their prediction intervals were estimated from own meta-analyses of literature and were benchmarked with existing meta-analyses of other food commodities. In addition, experimental validation data were obtained. The final outcome was a model to predict how many people can get ill by consuming spices and herbs. Some examples of specific pathogens in spices were used as illustration to show how the data can be used to get the best estimation, but also how variability impacts the outcome as well as various scenarios.

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## 2.15 Available community tools for predictive modelling

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Software tools are getting more and more important for decision-support in food industry and governmental agencies. This talk gives an overview on software resources in the domain of predictive modelling, summarises recent trends in software development and points to still unresolved challenges for software developers and end users.
The strategy developed and implemented by BfR in this area is showcased by two independent solutions from BfR's "FoodRisk-Labs" collection of open source software tools:

1) PMM-Lab – a software that facilitates the generation and deployment of mathematical models predicting growth or inactivation of microorganisms in food or feed matrices. PMM-Lab can easily be adapted to related disciplines, e.g. yield optimisation, toxin formation, dose-response prediction etc. With this flexibility PMM-Lab is one of the few generic open-source software solutions enabling food safety professionals as well as public health authorities to apply state-of-the art mathematical modelling concepts to their own or publicly available data.
PMM-Lab extends the KNIME framework (www.knime.org) through specific data processing functionalities (nodes) structured inside the PMM-Lab node library. These nodes allow users to apply or customise preconfigured workflows that create, visualise, analyse, save, import, export and deploy predictive (microbial) models or data. Moreover, consistent management of experimental data as well as predictive models is facilitated through the integrated preconfigured database. PMM-Lab can easily be customised, e.g. to perform product-specific shelf life or spoilage predictions, optimise food composition, food packaging or process conditions.

2) openFSMR – a web-based, community driven information portal on predictive food safety models. openFSMR guides users to available predictive food safety models which are either available in a standardised file format (PMF-ML) or which are implemented into specific software tools. The user interface has been designed to allow easy and fast information retrieval according to domain-specific search criteria including a full text search on all model metadata.
Technically openFSMR comprises of two components:
(a) a tabular data collection (openFSMR-DB) on food safety models implemented inside "Google Sheets". Relevant metadata were collected for each model and stored in the openFSMR-DB. All users are allowed to propose updates or corrections to the openFSMR-DB, while the DB admin can add new models to the database.
(b) a website to view, search, and select models inside the openFSMR-DB and to link out to the corresponding resources (tools or files). The website has been implemented using the free of charge software "Google Sites". The openFSMR website provides users also a functionality to send new models including their metadata to the admin of the openFSMR-DB.

All FoodRisk-Labs software tools and community resources can be accessed from the website https://foodrisklabs.bfr.bund.de.

## 2.16 Quality assurance of spices and herbs in official control

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The major objective of official food control is consumer safety and hence the detection of health-relevant constituents, contaminants and residues is important. Furthermore, the consumer must be protected from adulteration and intentional fraud as well as from deception (such as mislabelling). All aspects are important for a holistic control in the field of spices and herbs. In this keynote lecture, the general procedures of official control will be presented, with saffron as application example.

In Germany, 5 food samples per 1,000 inhabitants have to be sampled and analysed by official food control. The sampling is conducted by food inspectors on the local level and the analysis is typically conducted in centralised state laboratories, such as in the central spice laboratory at the CVUA Karlsruhe for the state of Baden-Württemberg. In 2014, 6981 samples of spices and herbs were analysed in Germany, from which about 10 % (699 samples) were found to be in offence against the food law. Most of these offences were labelling issues (588 samples), while only few samples were found to contain microbiological contamination (40 samples), other contamination (54 samples) or problems in composition (52 samples) [1]. The low incidence of serious problems can be partly explained by the EU system of border controls and rejection. For herbs and spices, the EU RASFF system [2] lists 147 notifications for 2015, 73 of which were border rejections due to microbiological contamination, mycotoxin contamination as well as exceedance of pesticide levels.
The official analyses in the field of spices and herbs are based on German national guidelines (so-called “Leitsätze”) as well as on ISO, EN and DIN norms. Generally, procedures such as ash, essential oils by hydro-distillation, macroscopic and microscopic examination and sensory testing are being conducted for all samples. Additionally, microbiological testing and verification of absence of residues and contaminants such as pesticides and mycotoxins is performed. Irradiation of spices and herbs is a permitted treatment which has to be labelled and is being routinely tested. During recent years a decline of irradiation of herbs and spices has been observed. More recent advances include the application of nuclear magnetic resonance spectroscopy (NMR), e.g. for spectral fingerprinting in authenticity control [3] or for quantitative analysis.

Due to the rather large diversity in the different herbs and spices, a number of different analytical techniques is necessary for authentication, as will be presented in the following examples of saffron analysis.
Saffron, the dried stigmata of the plant *Crocus sativus* L., is a spice used for colouring and flavouring food. It is considered to be the most expensive spice which can be explained by the laborious way the stigmata have to be harvested. There is to be made a considerable profit by adulterating saffron by mixing it with other saffron plant materials such as flower petals and styles or other colouring plants such as safflower or turmeric. Another way of adulteration is to use artificial colours to falsify deteriorated natural material or complete imitation using coloured paper [4-6]. For this reason, not only the natural material must be authenticated (e.g. by analysing saffron-specific colouring agents) using techniques such as NMR but also artificial colours must be detected by chromatographic means, e.g. thin-layer chromatography or high-performance liquid chromatography. From 15 saffron samples (mostly from internet trade) presented to the CVUA Karlsruhe in 2015, 13 were confirmed microscopically and using NMR to consist of natural saffron material, but one sample was additionally coloured with tartrazine (E102). Two samples were complete frauds (coloured paper). Additionally, 8 samples were objected because of offences against food labelling laws.

[1] Jahresbericht 2014 der Bundesrepublik Deutschland zum mehrjährigen nationalen Kontrollplan nach Verordnung (EG) Nr. 882/2004; www.bvl.bund.de/SharedDocs/Downloads/01_Lebensmittel/06_mnkp_dokumente/mnkp_Jahresbericht_2014.pdf?__blob=publicationFile&v=2

[2] RASFF – the Rapid Alert System for Food and Feed. https://webgate.ec.europa.eu/rasff-window/portal/?event=searchResultList

[3] Monakhova, Y. *et al*., Journal of Analytical Chemistry (2013) 68(9), 755-766: Chemometric methods in NMR spectroscopic analysis of food products

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[6] Sobolev A. *et al*., Foods (2014) 3, 403-419: Saffron samples of different origin: An NMR study of microwave-assisted extracts

## 2.17 Detection of natural and accidental contamination of spices and herbs

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Spices and herbs are important food flavourings, colourants, dietary supplements in gastronomy, and have prominent antioxidant and antimicrobial roles in herbal medicine and cosmetics. However, the environmental pollution and chemical contamination during the growth and storage of agricultural products, especially harvested in tropical or sub-tropical conditions, represent a serious threat to human health. Therefore, the maximum residue limits are established in the EU legislation for several substances.

Within the current research project the research team of scientists from the Netherlands (RIKILT), Germany (BfR) and Latvia (BIOR) has elaborated and implemented several techniques in order to assess the contamination status of spices and herbs on European market. A total of 300 samples representing six condiments (black pepper, basil, oregano, nutmeg, paprika, and thyme) were analysed for 11 mycotoxins, 134 pesticides, and 4 heavy metals by ultra-high performance liquid chromatography–tandem quadrupole mass spectrometry and inductively coupled plasma mass spectrometry. 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 of pesticides were exceeded in 10 % of oregano and 46 % of thyme samples. A risk assessment of heavy metals was performed, indicating daily intake levels far below the tolerable intake levels.

The performance assessment of the Orbitrap-HRMS technology confirmed this methodology as a high-throughput technique providing concentration values in a good agreement with the results obtained by the classical triple quadrupole detection method. However, the higher resolving power of Orbitrap mass spectrometer showed a clear benefit by diminishing the signals of interfering compounds from spices matrices.

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## 2.18 A proposed comprehensive strategy to detect the fraudulent adulteration of herbs: The oregano approach

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Fraud in the global food supply chain is becoming increasingly common due to the huge profits associated with this type of criminal activity. Food commodities and ingredients that are expensive and are part of complex supply chains are particularly vulnerable. Both herbs and spices fit these criteria perfectly and yet strategies to detect fraudulent adulteration are still far from robust. An FT-IR screening method coupled to data analysis using chemometrics was shown to be 95 % accurate in detecting adulteration of oregano. A second method using LC-HRMS was developed which was able not only to detect and quantify the amount of oregano but also commonly used adulterants by biomarker identification. This confirmatory method was shown to be > 99 % accurate. The two-tier testing strategy was applied to 78 samples obtained from a variety of retail and on-line sources. There was 100 % agreement between the two tests that over 24 % of all samples tested had some form of adulterants present. The innovative strategy is now being applied as a basis for testing the global supply chain for fraud in oregano and could be the basis for a comprehensive strategy to detect fraud in many herbs and spices.

## 2.19 Deliberate contamination – Detection by spectrometric fingerprinting methods

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Spices and herbs are present in many foods, and especially in composite and processed foods. Spices are mostly grown in (sub)tropical areas and subsequently imported into the EU in crude, dried and/or ground form. These spices are processed and packaged for retail, but are also sold from business to business to be further applied in the industrial sector. Spices and herbs are a group of products reported very frequently in the food fraud databases. Due to their high price per kg, the long and not so transparent supply chain, as well as cultural and behavioural issues spices are fairly vulnerable to fraud. In the EU project SPICED we have been developing rapid anomaly testing methods for broad detection of this kind of frauds. The methods concern non-targeted fingerprinting methods using mass spectrometry in combination with statistical techniques. Pepper and nutmeg are the main spices focused on with this spectrometric broad anomaly testing approach. For both pepper and nutmeg addition of lower quality product own material is common, which should be declared if added on purpose. This group includes products such as peel, spent, light berries, spiral rejects, etc. Results from the spectrometry analysis applied in the project show that these products can be distinguished from the premium pepper and nutmeg products, also when present in mixtures. A survey shows the presence of the lower quality products in spices on the market.

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## 2.20 Deliberate contamination of spices and herbs – Detection by spectroscopic fingerprinting methods

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As spices and herbs are typically high-price commodities, fraudulent practices promise high economic profit. Therefore, it is not surprising that spices and herbs are among the top positions of reported cases of food fraud. One main objective of the EU funded project SPICED is to facilitate the proof of adulteration and to ensure the authenticity of spices and herbs by development of rapid and cost-efficient (high throughput) methodologies for the detection of natural, accidental and deliberate contamination of spices and herbs with chemical agents. For this purpose non-targeted analytical approaches are evaluated and improved. The so-called fingerprinting methods involve spectroscopic or spectrometric analysis followed by multivariate statistical data evaluation and aim to capture as many features or compounds as technically possible within one measurement to provide a comprehensive insight into the composition of the sample. Characteristic fingerprints of authentic (non-adulterated) sample sets are the basis for identifying misdescription or adulteration.

Representative sets of authentic paprika/chili, nutmeg and oregano samples were analysed by nuclear magnetic resonance (NMR) spectroscopy and Fourier transform infrared (FTIR) spectroscopy, respectively. The acquired spectroscopic data were used to:

(i) identify an appropriate data pre-processing for NMR and FTIR data, respectively,
(ii) determine the data space of non-adulterated samples by principal component analysis (PCA) and
(iii) develop a chemometric classification or outlier detection that allow distinguishing authentic samples from samples which are adulterated with lower quality material or chemical additives such as dyes.

The basic ideas and results of the study will be presented.

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## 2.21 New approaches for the decontamination of spices and herbs

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The increasing consumer demand for minimally processed and ready-to-eat dishes cause new challenges with regards to the microbial safety of spices and herbs. Especially the surface of whole black pepper is often spoiled with a high load of bacterial endospores. The traditionally applied technologies to decontaminate these dry products often cause quality losses or are rarely accepted by the consumers.

To ensure the microbial safety and preserving the quality of spices the application of selected novel technologies was investigated within the European research project “GreenFooDec” (FP7-SME-2011-285838).

Among others, cold atmospheric plasma is a promising nonthermal technology. The process enables a microbial multi-target inactivation process and the high diffusion rate of the plasma allows a treatment of non-uniformly shaped products. Hence the antimicrobial effect of direct and indirect plasma for the decontamination of herbs and spices will be presented.

As an example whole black pepper seeds were inoculated with *Bacillus subtilis* and *Bacillus athrophaeus* spores to achieve a contamination level of approximately $5 \times 10^7$ CFU/g. For the direct plasma treatment, the argon gas afterglow of a radio-frequency plasma jet was used. The plasma gas for the indirect treatment was generated by a microwave plasma set-up with air as feed gas. During the treatments the surface temperature of the pepper remained constant at 22 °C during the indirect plasma treatment and increased up to 55 °C during the direct plasma application (after 15 min). To quantify the quality of the treated pepper, colour and weight changes were measured. The main aroma compound of pepper, piperine, was measured by HPLC.

The direct plasma treatment achieved a rapid inactivation of 1.61 $\log_{10}$ (*Bacillus subtilis*) within the first 5 min and 2.85 $\log_{10}$ after 15 min. All inactivation kinetics were biphasic, pointing towards a rapid UV light inactivation followed by slower inactivation caused by photodesorption and etching. Minor impact on the product colour and the piperine content (-6.3 %) was detectable, but a weight loss of 5.2 % was obtained. The indirect plasma treatment rarely affect the pepper quality and reduced the colony counts for the natural pepper flora, *Bacillus subtilis* and *Bacillus athrophaeus* by 2.21 $\log_{10}$, 2.28 $\log_{10}$ and 3.18 $\log_{10}$ after 30 min exposure time. A treatment of slightly humidified pepper further enhanced the inactivation. However, the main challenges in plasma processing of food materials are: i) proper selection of the plasma source, ii) characterisation of product-process interactions including quality and safety attributes, and iii) optimised process design and up-scaling for industrial application. Recent developments will be discussed.

## 2.22 Decontamination of spice paprika

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One of the tasks of the EU-FP7 project SPICED is the evaluation of available means and techniques for the decontamination of spices. As irradiation of spices has been approved by the European Food Safety Authority (EFSA) as being a safe treatment method, it has been to be compared 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.

For our experiments, spice paprika powder samples have been irradiated with ionising radiation ($^{60}Co$) with 1, 5 and 10 kGy in the Institute of Isotopes Co., Ltd, Budapest, Hungary. Our results have confirmed that the microbial contamination of spice paprika powder can be effectively reduced by ionising radiation. Following irradiation treatment the initially dominant microflora of bacilli (*B. methylotrophicus*, *B. pumilus*) gradually disappears, and species less sensitive to irradiation (*Methylobacterium* spp., *Micrococcus* spp., and *Microbacterium* spp.) come into view.

Steam treatment (saturated dry steam, 108–125 °C for 20–120 sec) reduced mesophilic aerobic total bacterial count from $1.8 \times 10^5$ CFU/g to $6.0 \times 10^2$ CFU/g, and moulds from $1.3 \times 10^2$ 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 was performed in a Daewoo Kor-630A laboratory equipment. No relevant reduction of the mesophilic aerobic total bacterial count could be observed following the treatment at the parameters studied. The colour of the paprika powder got darker and had a brownish character.

Radio frequency treatment was done by Laboratory equipment with 10 kW Brown Boveri generator, 13.5 MHz. The microbial load of the samples showed no reduction even for the most severe treatment. The colour of all treated samples were significantly darker than the control, they had a burnt character.

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## 2.23 Import control of food of non-animal origin

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Whereas the import control procedures of products of animal origin have been established quite early and are regulated in detail within the EU, the import controls of food of non-animal origin have been put in place mainly after the year 2000 and are still dominated in many ways by national provisions in the different EU-Member States.

Currently we can find a number of safeguard measures like Regulation (EU) No. 884/2013 with regard to control of aflatoxins or Regulation (EU) No. 669/2009 laying down certain control measures and procedures which are accompanied by the general control provisions of Regulation (EC) No. 882/2004.Therefore we can distinguish so far between a control regime at the external EU border for food products within the scope of re-enforced controls and a general control system for all other food of non-animal origin which is set according to Article 15 (1) of Regulation (EC) 882/2004.

The regime of re-enforced checks, meaning the controls established by Regulation (EU) No. 669/2009 and most of the safeguard measures only permits the entry of products via designated points of entry (DPE) at an EU border which means at ports, airports or land borders. The arrival of a consignment in the EU has to be pre-notified to the competent authority of the DPE and has to undergo there at least a documentary check. Pursuant to a determined frequency an identity and physical check including a laboratory check are performed. Under certain conditions an onward transportation to the designated point of import (DPI) can be granted before the laboratory result is received. The final decision of the official food control has to be stated on the Common entry document (CED) which is an obligatory EU document for the customs authorities to release these food consignments for free circulation in the EU. Nevertheless despite the establishment of EU wide procedures problems have been recognized in specific areas, e.g. arbitral sampling or the onward transportation.

Due to the extensive checks in the area of re-enforced controls it may be easily overlooked that the majority of food of non-animal origin from third countries do not undergo a regular import control in several EU countries. The requirements of the EU control regulation foresees risk based checks which can be performed at any stage of the food chain from the entry into the EU, during storage or at the retail level. As there is only a general framework set by EU legislation there are distinctive differences in the control level of food of non-animal origin varying from a regular import control for all kind of food products like for instance in Spain, over targeted checks at the time of import to checks mainly at the retail level. Therefore an EU wide risk assessment of all imported food of non-animal origin can be hardly achieved. This is even more relevant if the system of pre-export checks in third countries is taken into account.

## 2.24 Overview on the Rapid Alert System for Food and Feed (RASFF)

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The Rapid Alert System for Food and Feed (RASFF) was established in 1979 as a tool for the competent authorities of the European member states to exchange information in a fast and structured way concerning measures taken responding to serious risks identified in food or feed. To ensure the effective exchange of information all the members of the network (EU Member States, European Commission, EFSA, EEA) have designated contact points and communicate within the network by means of predefined templates. Legal basis of the RASFF is Article 50 of Regulation (EC) No 178/2002. In addition Regulation (EU) No 16/2011 with implementing rules for the RASFF entered into force on the 31 January 2011.

Since its establishment the RASFF system has been improved and has progressed in many ways. Starting with fax messages and telephone calls 37 years ago, nowadays the members of the network communicate within an online application, the (interactive) RASFF.

In the year 2015, 3049 original notifications were transmitted and gave rise to 6204 follow-up notifications. The most notified hazards were pathogenic microorganisms (745 notifications), mycotoxins (495 notifications), pesticide residues (405 notifications) and heavy metals (219 notifications). 150 notifications concerned the food category herbs and spices. In 30 cases the presence of pathogenic microorganisms led to an alert in the RASFF. In the majority of these cases *Salmonella* spp. were detected.

## 2.25 Forward-backward tracing with FoodChain-Lab: Software supporting foodborne disease outbreak investigations

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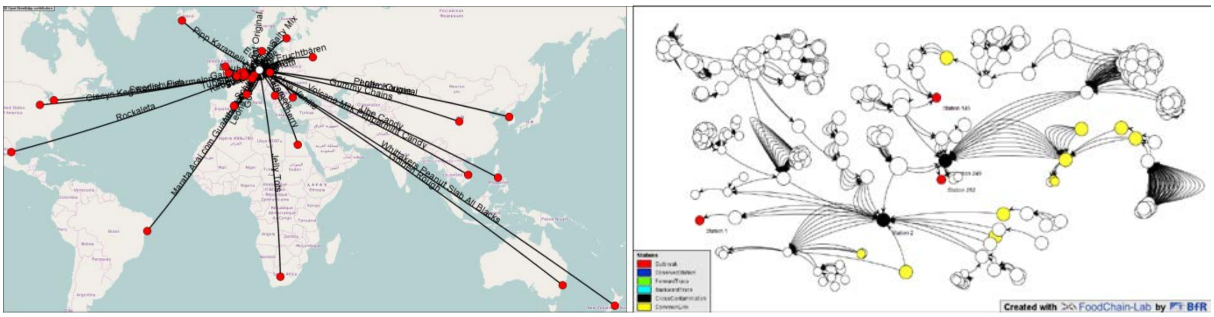
In case of foodborne disease outbreaks, rapid identification of the causative food product is essential, since the medical and economic damages grow with the duration of the outbreak. Recent foodborne disease outbreaks in Europe illustrated that there is a need for an expert software system capable of supporting investigations on supply chains as well as exposure assessments in crisis situations. Furthermore, the expert software system should be able to provide a comprehensive data management infrastructure assuring highest possible data quality and integrity at any point in time.
To address these needs a free, open source software called FoodChain-Lab has been developed.

Since its initial application during the EHEC outbreak in Germany in 2011 FoodChain-Lab has been used and tested in several outbreak investigations, e.g. the Norovirus outbreak in Germany in 2012 or the Hepatitis A outbreak in Europe. On the basis of these experiences the software evolved from a data visualisation and analyses tool into a comprehensive tool box for data management, data enrichment, visualisation, data analysis and interactive reasoning.

FoodChain-Lab is applicable in feed or foodborne disease outbreak investigations as well as in exposure assessment tasks related to feed or food supply chains.
FoodChain-Lab has been implemented as an extension to the modular open source data analytics platform Konstanz Information Miner (KNIME). KNIME enables visual assembly of data analysis workflows.

The installation guide, the source code, example workflows and sample data are available via https://foodrisklabs.bfr.bund.de.



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**Weiser *et al.*, 2016**: “FoodChain-Lab: a trace-back and trace-forward tool developed and applied during food-borne disease outbreak investigations in Germany and Europe”, **PLoS ONE**

## 2.26 Recommendations for monitoring the most relevant hazards in the spice and herbs chain

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**Introduction**: The European Union (EU) is an important market for exporters of spices and herbs. As high quality and food safety are at the forefront, monitoring is critical. The need for an effective monitoring system is even more pertinent given the extensive nature of the spice and herb supply chain and the various hazards that may occur.

**Purpose**: The aim of this study was to design a monitoring model to select most relevant chemical and biological hazards-product combinations, which could be used to provide recommendations for monitoring.

**Methods**: Dutch and German monitoring systems for spices and herbs were compared and evaluated. Rapid Alert System for Food and Feed (RASFF) notifications, data from Eurostat and EU pesticide databases, and expert opinions on the relevant hazards were also collected. A Bayesian Network (BN) method was used to develop a model, based on these data, aiming to predict the most relevant hazards, products, and origin countries to monitor at several points in the spice and herb supply chain.

**Results**: Results indicated that the monitoring approach for a particular point in the chain depends on the product-hazard-origin combination. For example, at border inspection, the controller should focus on monitoring curry leaves, chili pepper, and curry. The main chemical hazards detected were aflatoxins (27 %), ochratoxin A (9 %), and Sudan I (8 %). At the market, the controller should focus on paprika, chili pepper and nutmeg. The main chemical hazards detected were aflatoxins (32 %), ochratoxin A (14 %), and Sudan I (5 %).

**Significance:** This model can aid governmental authorities and industry in defining which combinations of products, hazards, and origins to monitor at different stages along the spice and herb supply chain.

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# 3 Poster Abstracts

## 3.1 Food fraud vulnerabilities in the spices chain

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In the past decades, the quality control of imported spices changed tremendously. Previously, employers were trained to check the spices visually and by nose to rate the raw material quality attributes. Nowadays, control measures have become much more strict and spice manufacturers also outsource the quality checks to specialised laboratories. In addition to quality and safety aspects, awareness of food fraud has raised in the past few years and spices are among the most fraud vulnerable product groups world-wide. Recent scandals have increased the need to strengthen companies' ability to combat fraud within their own organisations and across their supply chain but current food safety management systems (FSMS) are not specifically designed for fraud detection or mitigation. A first step to include measures in FSMS and to carry out adequate fraud vulnerability assessments is the identification of relevant risk factors. We have identified key food fraud risk factors based on the principles of the criminological routine activities theory which takes into account a suitable target (opportunities), a motivated offender (motivations) and (lack of) guardianship (controls). This concept has been developed into a food fraud vulnerability self-assessment tool commissioned by and in collaboration with SSAFE (a collaboration with multinationals to ensure consumer's food safety). This questionnaire is accessible for all interested companies via an online tool that PwC developed together with SSAFE (http://www.pwc.com/foodfraud).

In this study the vulnerability to fraud of companies along the spices supply chain was assessed using the approach mentioned above and generic vulnerability profiles were established. For the assessment of the vulnerabilities in the spices chain, several actors were interviewed, from the importer to the companies that develop consumer products. The basis for these personal interviews are the questions from the food fraud self-assessment tool that was mentioned before. The questionnaire contains 50 closed questions, divided into opportunities, motivations and control measures. With the answers, we were able to profile the companies' strengths and weaknesses. The interviews focused on one single spice, *i.e.* white pepper. Especially the opportunity risk factors indicate the vulnerability of the spices. The knowledge on how to adulterate is quite common and since spices are milled, it is harder to find potential adulterants, whereas the technology is widely available. The motivations and control measures vary more along the chain.

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## 3.2 Detection of spice adulteration using non-targeted spectroscopic fingerprinting techniques

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Securing the food chains from primary production to consumer-ready food against any kind of contamination and/or adulteration is a prerequisite for food safety. Spices and herbs are among the top notifications in the European Rapid Alert System for Food and Feed, which indicates the need for dedicated controlling this commodity. Within the EU project SPICED, among others, fingerprinting techniques are evaluated and improved to ensure the authenticity of spices and herbs. Furthermore, approaches for identifying chemical hazards within these commodities are developed.

Non-targeted analytical methods aim to capture as many features or compounds as technically possible within one measurement and to obtain a comprehensive insight into the composition of food samples. In this study, paprika, black pepper, basil and oregano samples were investigated to (i) determine the data space of non-adulterated (authentic) samples and (ii) identify addition of lower quality material or adulterants such as dyes. For this, the spectroscopic fingerprints of these spices and herbs were detected by nuclear magnetic resonance (NMR) spectroscopy and subsequently analysed by principal component analysis (PCA). Basil samples resulted in a smaller variation than pepper and paprika samples based on, for example, non-polar NMR measurements. Furthermore, paprika samples adulterated with beetroot could be clearly separated on the basis of NMR measurements and PCA evaluation. In short, the exemplarily presented results demonstrate the potential of spectroscopic analysis to determine adulterations of spices and herbs.

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## 3.3 Validation and reporting standards of non-targeted fingerprinting approaches for food authentication

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Fingerprinting approaches are expected to become a potent tool in food authentication processes aiming at a comprehensive characterisation of complex food matrices. By non-targeted spectrometric or spectroscopic analysis and subsequent multivariate statistical evaluation, food matrices can be investigated in terms of their geographical origin, species variety or possible adulteration. Although many research projects have already demonstrated the feasibility of non-targeted fingerprinting approaches, their uptake and implementation into routine analysis and food surveillance is still limited. Regarding the huge amount of data that is generated in the cause of fingerprinting studies, challenges that have to be faced are to (i) distinguish study-relevant information from unintended systematic variation and noise and (ii) guarantee the robustness of the extracted data.

A review of literature with regard to validation strategies for food authentication using metabolic fingerprinting techniques revealed two issues that may hinder a broader application of such approaches: a lack of validation strategies for the metabolomics workflow and limited reporting on data quality.[1] In particular, model validation within the experiment was often well addressed, but the accuracy of the results considering experiment-to-experiment, instrumental, operator or laboratory variation was hardly considered. Moreover, data processing was little transparent in many studies with poorly characterised metabolic fingerprints used for data evaluation. On this basis, recommendations and future work for validation as well as reporting of non-targeted food fingerprinting are derived to progress standardisation for a potential implementation of such approaches in routine analysis for food control purposes. These suggestions of ‘good practice’ for validation and reporting might also be relevant for further applications within regulatory frameworks.

Acknowledgement: This research was executed in the framework of the EU project SPICED (Grant Agreement: 312631) with the financial support from the 7th Framework Programme of the EU.

[1] J. Riedl, S. Esslinger, C. Fauhl-Hassek, *Analytica Chimica Acta* 2015, 885, 17-32.

### 3.4 A fast dilute-and-shoot method for the determination of 30 pyrrolizidine alkaloids and related N-oxides in plant materials using LC-Q-Orbitrap-MS analysis

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Pyrrolizidine alkaloids (PAs) are a group of food contaminants which are exclusively biosynthesised by plants. So far more than 600 different PAs and PA N-oxides are known and it has been estimated that approximately 6000 plant species, representing 3 % of all flowering plants, are able to express PAs. Typical plant families are Boraginaceae, Asteraceae and Fabaceae. PAs are derived from the chemical structure of pyrrolizidine and can be subgrouped into retronecine-, heliotridine-, crotanecine-, supinidine- and otonecine-type PAs. In particular, the 1,2-unsaturated compounds are known to expose hepatotoxic effects and they are under suspicion to be genotoxic and carcinogenic. Common sources for PA intoxications of humans and livestock are phytopharmaceuticals, food and feed supplements, herbal teas, milk, honey and silage. In these commodities high concentrations of up to several mg/kg can be found. So far, no regulatory limits have been defined for this group of contaminants in food and feed. On this poster a fast dilute-and-shoot method followed by LC-Q-Orbitrap analysis for PA identification and quantification in plant materials will be presented. The sample preparation was carried out by a liquid-solid extraction of homogenised sample materials using a mixture of acidified water/methanol followed by shaking, centrifugation and dilution of the supernatant. For analysis, an Ultimate 3000 UHPLC system coupled to a Q-Exactive Orbitrap mass spectrometer was used. The mass spectrometer was operated in fullscan + data dependent MS2 acquisition mode (ESI+) with a resolving power of 70,000 FWHM. The analytes were identified by verification of their exact mass, isotopic pattern and MS/MS fragmentation. As there are no internal standards available on the commercial market so far, quantifications were performed using matrix matched calibrations. In order to assess the fitness-for-purpose of the method a validation was performed by PA fortification of blank herbal tea extracts in a concentration range of 0.5–10 ng/mL (representing 10–200 ppb sample concentration). The linearity was found to be very good with $r^2 \geq 0.9994$ for 29 out of 30 compounds. The precision and LOQs of the method was determined by a six fold determination of fortified extracts at 10 ppb and 40 ppb. The results show an RSD of $1.4 \pm 0.7$ % and LOQs of $2.6 \pm 1.2$ ppb. Further performance parameters regarding the analytical method will be presented on the poster.

## 3.5 Culture-independent detection and quantification of G- and G+ pathogenic bacteria in spices and herbs

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Our preliminary experiments using standard culture-based methods for detection or quantification of pathogenic bacteria in spices and herbs produced compromised values of limit of detection (LOD) or limit of quantification (LOQ). Antibacterial activity of the compounds contained in the matrices caused growth inhibition in enrichment media for detection of low numbers and on agar media for enumeration of bacteria. As an alternative, culture-independent approach was developed in this study. For this purpose, DNA extraction was optimised by testing various methods based on chaotropic solid-phase extraction (various kits) or liquid-liquid extraction. DNA extraction method using CTAB, primarily developed for plant DNA extraction, facilitated co-extraction of bacterial DNA of required concentration. The extracted DNA was downstream analysed by real-time PCR. Detection and quantification parameters of the culture-independent approach were evaluated with black pepper and paprika (representatives of spices), and parsley and oregano (representatives of herbs), which were artificially contaminated with *Salmonella enterica* (Gram-negative representative) and *Staphylococcus aureus* (Gram-positive representative). Results demonstrated quantitative response with linear calibration lines, with LOQs of $10^3$ CFU/g (for all analysed matrices) and LODs of $10^2$–$10^3$ CFU/g. The presented method facilitates reliable direct detection and quantification of the pathogens in spices and herbs within eight hours.

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## 3.6 Detection of viable *Escherichia coli* in dried herb and spice matrices by FCM analysis

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Herbs and spices are natural products used to flavour and colour many food products. They are cultivated in many countries, mainly in areas where sanitation conditions are generally poor. Despite the biological growth-inhibiting dryness of herbs and spices, they are natural products harbouring a wide range of microorganisms. Natural, accidental or deliberate microbial contaminations can take place at numerous vulnerable points (harvesting, handling, transportation), and could reach up to $10^8$ CFU/g. This could lead to various foodborne infections and intoxications of consumers.

Traditional bacterial cell-culture methods used to detect and identify pathogens involve multiple procedural steps that can take up to a few days. In order to increase customer health and safety, it is crucial to develop fast and economic methods to detect microorganisms.

Since flow cytometry (FCM) is emerging as an alternative method of rapid real-time microbial detection, this technique was employed to detect viable *E. coli* cells in 5 herbs and spices. The chosen condiments included dry powdered basil, oregano, black pepper, cinnamon and nutmeg. As no *E. coli* was detected in any of the tested herbs and spices, and microbial content did not exceed $10^4$ CFU/g, the condiments were artificially contaminated with live *E. coli* at ~$10^7$ CFU/g. Next, a sample clean-up procedure was performed which included dilution, stomaching, a range of filtrations, low and high speed centrifugations, washes, vortexing and density gradient centrifugation. The procedure resulted in the removal of food matter which could clog the flow cell of the cytometer. More importantly, it resulted in the recovery of bacteria from the matrix particles, the sizes and fluorescence of which can also interfere with the bacterial detection.

The sample preparation and gating strategy resulted in a distinct separation of live/intact *E. coli* cells from these 5 matrices when spiked with ~$10^7$ CFU/g of target bacteria. Recovery of bacteria occurred at the level of ~$10^7$ CFU/g, which was confirmed by enumeration of events in the live population region of the cytograph as well by traditional plate counting. Furthermore, analysis of unspiked matrices revealed that the Percoll layer characteristic for *E. coli* cells contained less than 20 cells arising from background microflora. Events from distinguished (live, dead and matrix) populations of pepper samples spiked with *E. coli* were sorted from each region using Fluorescence Activated Cell Sorting (FACS) and plated on to APC and TBX agars. As expected, no growth was observed on plates from events sorted from dead and pepper matrix regions. Colony counts on APC and TBX agars were in agreement with the number of events sorted from the live region.

The clean-up procedure preceding FCM analysis allowed for successful recovery and separation of *E. coli* cells from the food matrix and from background microflora in 5 herbs and spices. Furthermore, live/dead staining and multiple gating procedures resulted in a distinct separation of live *E. coli* cell sub-populations from the residual matrix particulates in the condiments. The developed FCM method can deliver results within 2–3 hours.

Acknowledgement: This research was executed in the framework of the EU project SPICED (Grant Agreement: 312631) with the financial support from the 7$^{th}$ Framework Programme of the EU.

## 3.7 Differentiation of the bacterial living and non-living community in the heterogeneous matrices of spices and herbs

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Spices and herbs are contained in almost every processed food, including ready-to-eat products. Further, they are natural products that can be contaminated with several microorganisms, and therefore can cause food-borne diseases. The rapid identification of condiments as cause of an outbreak can be difficult, as consumers and experts often focus on major food ingredients instead of minor components. In general, securing the food chains from primary production to consumer-ready food against major deliberate, accidental or natural contaminations is directly related to the safety of food products.

In this context the differentiation of the living from the non-living bacterial community in food including the complex matrices of spices and herbs is of importance. Therefore we used live-dead staining with the LIVE/DEAD® BacLight™ Kit from Invitrogen (Thermo Fisher Scientific, USA), followed by analysis with fluorescence microscopy. For this purpose the Gram-negative *Escherichia coli* and the Gram-positive *Staphylococcus aureus* and *Bacillus atrophaeus* were used as indicator test organisms. The test organisms were mixed into the different matrices of spices and herbs and grown up to the late-log-phase. Then the samples were harvested, stained and analysed by fluorescence microscopy. The organisms showed in some matrices lower growing rates. Furthermore, some of the different types of spices and herbs have had negative effects on the performed staining method. The natural cell morphology of the indicator test organisms growing in spices and herbs did not change during the experiments. This is a major advantage of using the staining method, because the natural cell morphology of organisms, for example coccoid structures of rod-shaped forms are distinguished and did not change with this technique.

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### 3.8 Occurrence and diversity of moulds in spices and herbs

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Spices and herbs consist of several anatomical parts of the plant, which are not of high nutritional value, however they have great sensory importance and influence on the digestive process (Garbowska *et al.* 2015). Nevertheless, spices and herbs can contain spore-forming bacteria, such as *Bacillus cereus*, Enterobacteriaceae, and moulds, such as *Aspergillus*, *Penicillium*, *Fusarium* spp. (Ainiza *et al*. 2015). Mycotoxin-producing moulds can cause health problems for consumers, as well as contribute to food spoilage and reduce shelf-life (Prakash *et al.* 2015).

The aim of this study was to determine microbiological contamination in spices and herbs, collected from various retail points.

Overall, 150 samples of 27 different types of spices, including black ground pepper, cinnamon, curry and others, and 45 samples of 11 different herb types, including basil, rosemary and others, were tested for the presence and diversity of moulds. Samples were obtained from supermarket chains and local markets. Presence of moulds and water activity were tested by ISO standards. Mould species were determined by the Matrix Assisted Laser Desorption/Ionisation method.

Our results revealed that moulds were found in 121/150 (81 %) samples. Number of moulds varied between 1 $\log_{10}$ CFU/g to 4.68 $\log_{10}$ CFU/g. The highest number of moulds was found in minced ginger 4.48 $\log_{10}$ CFU/g. Type of commercialisation (a supermarket chain or market) significantly affects the microbiological contamination ($p < 0.05$) in spices and herbs. Water activity ranged from 0.36 to 0.67 and it had a low correlation with the level of microbiological contamination. Overall, 8 genera of moulds were found, including *Aspergillus* spp. (45 %), *Fusarium* spp. (31 %), *Penicillium* spp. (10 %), *Alternaria* spp. (5 %), *Mucor* spp. (3 %), *Cunninghamella* spp. (2 %), *Cladosporium* spp. (2 %) and *Stemphylium* spp. (2 %). Samples from the market showed higher prevalence and diversity of toxin-producing fungi, including *A. flavus*, *A. ochraceus*, *A. versicolor* and *A. nomius*, which suggests that trade type not only effects the fungal outgrowth, but also can be linked to its potential for causing severe health problems.

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## 3.9 Antimicrobial activity of spices and herbs against *Salmonella* spp.

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In general, dried spices and herbs are considered to be safe food ingredients by common people. However, especially because condiments are often used directly at the table or for ready-to-eat foods where no final germ reduction step is applied, this is not the case. According to the notifications and alerts at the Rapid Alert System for Food and Feed, spices and herbs are placed within the top five ranked matrices where *Salmonella* spp. is the highest ranked pathogen. Although *Salmonella* spp. do not produce spores, they are able to persist for months up to years. Thus, they rely on completely different mechanisms to allow resistance as for example accumulation of osmotic active substances or entering a viable but nonculturable state.

In the present study inocula of *Salmonella* Oranienburg (originally isolated from ground cumin) were produced for spiking nine spices and herbs. Subsequently, a screening for potential antimicrobial activity of the condiments against *S.* Oranienburg in a 1:10 (w/w) dilution with buffered peptone water was performed during incubation for 72 h. The effects were monitored with an enumeration of the colony forming units (CFU) as well as a qualitative approach. In addition, a tenacity study was set up where the dry condiments were spiked with dried *S.* Oranienburg.

To sum up, oregano and cinnamon completely inhibited not only the proliferation of *S.* Oranienburg but also showed a bactericidal effect, while for thyme and allspice adaptations might have been apparent, which resulted in a recovery from temporal inhibitions. However, five matrices showed no remarkable antimicrobial activity compared to the control.
The survivability of *S.* Oranienburg during the dry storage at 25 °C was significantly influenced by the matrix where a higher susceptibility towards spices than herbs was obvious. Especially paprika/chili showed a significant reduction of recovery after storage for a half year, followed by allspice and nutmeg, while the difference in recovery in cinnamon, pepper, thyme, basil and parsley was $\log_{10} < 0.5$ CFU $g^{-1}$ matrix, compared to the control. Oregano led to a completely different trend with an already reduced recovery after spiking, however, no significant reduction was detected during the storage.

Hence, the antimicrobial activity of several condiments was confirmed, in suspension as well as in a dried form, which emphasises the necessity for further improving the ISO 6579 method for the detection of *Salmonella* spp. in condiments.

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### 3.10 *Clostridium perfringens*: Antimicrobial activity of spices and herbs and factors influencing the spore production

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Although average persons do not raise concerns over microbial risks and food safety issues while handling with the small dried ingredients spices and herbs, they harbour a significant health risk potential. *Clostridium perfringens* is a relevant pathogen with respect to food-borne outbreaks affecting the intestinal tract causing vomiting, diarrhoea, abdominal pain, and dehydration. Due to their capability to change from the vegetative form into a dormant spore, they are resistant against various harsh environmental conditions and treatments, and thus are predestined to persist within these matrices for several years and more.

In the present study within the EU project SPICED, investigations were performed with respect to transfer *C. perfringens* into the spore form. For that, several aspects were taken into account, as they were (i) strain-specific spore production capacity, (ii) optimisation for cultivation and enrichment, (iii) induction for germination with heat treatment, as well as (iv) effect of supplementation of germination factors.

In short, three different *C. perfringens* strains were compared regarding their ability to grow and sporulate in several different growth media, as well as their capacity to produce significant numbers of heat-resistant spores. Furthermore, the heat treatment for germination resulted in a significant increase in colony forming units (CFU). Thus, it was a mandatory step for recovery of the actual CFU. However, the supplementation of germination factors did no lead to an additional increase of spore CFU of the three investigated strains.

Last but not least, potential antimicrobial effects of nine condiments samples (five spices and four herbs), were determined and compared to the control. In addition, the CFU determination was done prior and after heat treatment with 80 °C for 10 min. Interestingly, no condiment showed antimicrobial effects prior heat treatment, while rather beneficial effects were determined. However, after the treatment, the recovery of *C. perfringens* in cinnamon and allspice was significantly reduced. Thus, the heat might have led to an increased extraction of potential antimicrobial compounds within these two matrices, inhibiting either the germination of the spores or the subsequent growth of vegetative *C. perfringens* cells.

To conclude, the present study led to a further understanding of the sporulation strategies of *C. perfringens*, its detection, as well as the potential effects of condiments.

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### 3.11 Inoculation techniques affect the survival of *Brucella microti* in dried herbs and spices

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**Background:** As flavouring and seasoning ingredients, herbs and spices are added to various foods; however, they can be contaminated with pathogenic microorganisms. Despite possible antimicrobial properties, contaminated condiments can be a source of foodborne illness outbreaks. Therefore, studies on survival of microorganisms in condiments are of relevance for microbial food safety risk assessment.

*Brucella* (*B.*) *microti* was initially isolated from common voles (*Microtus arvalis*) and later also from soil. Hence, *B. microti* may be able to survive in natural environments. Its virulence for humans is hitherto unknown. To get a deeper insight into the potential role of *B. microti* as a contaminant of plant-derived food products, we investigated its survival rates in dried condiments, which were artificially contaminated by several inoculation techniques.

**Material and methods:** Dried and ground oregano, pepper, paprika and parsley were inoculated with *B. microti* using one wet inoculation technique and several dry transfer techniques. Liquid cultures were prepared by loosening two-day old *B. microti* lawns in either Brucella broth, milk powder suspension (2 %, v/w) or lyophilisation medium. Transfer was carried out as follows:

1) a) Sand-spiking B: bacteria in *Brucella* broth were air-dried on sand, then added to the condiment (final cell count after drying: 6.68 ± 0.21 $\log_{10}$ CFU/g)
   b) Sand-spiking M: bacteria in milk powder suspension were air-dried on sand, then added to the condiment (final cell count after drying: 6.29 ± 0.30 $\log_{10}$ CFU/g)
2) Wet-spiking: bacteria in *Brucella* broth were air-dried directly on the condiment (final cell count after drying: 5.87 ± 2.06 $\log_{10}$ CFU/g)
3) Lyo-spiking: bacteria in lyophilisation medium were freeze-dried, crushed to powder and added to the condiment (final cell count after drying: 9.85 ± 0.36 $\log_{10}$ CFU/g)

Samples were stored in the dark at room temperature. All experiments were repeated three times in parallel. Cell counting of *B. microti* was conducted in duplicates (detection limit: 1.3 $\log_{10}$ CFU/g) at following days after inoculation: 0, 3, 7, 10, 14, 21, 28, 42, 56, 84 and 112.

**Results:** The inoculation process showed a greater effect on the survival of *B. microti* than various condiment matrices. After 16 weeks of storage, survival of *B. microti* was most stable by using the lyo-spiking technique with a mean survival rate ranging from 5.04 to 7.04 $\log_{10}$ CFU/g, followed by sand-spiking M (2.74−4.06 $\log_{10}$ CFU/g), sand-spiking B (1.00−2.48 $\log_{10}$ CFU/g) and wet-spiking. By using the latter technique, viable bacteria could not be detected anymore in oregano and paprika after six weeks and in pepper and parsley after 16 weeks.

**Conclusions:** In the natural environment, *B. microti* can be transferred to condiments. Our inoculation techniques may mimic several routes of contamination; e.g. sand-spiking may represent contamination by dust and soil particles. Our results indicate that the way of contamination affects the survival of *B. microti* in condiments. In addition, compounds of the carrier fluid can also play a crucial role in maintaining viability during storage. Also the condiments themselves may due to antimicrobial compounds harm *B. microti* during inoculation as well as during storage. In conclusion, under environmental and transfer conditions comparable to our study design, *B. microti* can survive for more than 16 weeks in paprika, pepper, oregano and parsley.

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### 3.12 Survival of pathogenic microorganisms in spices and herbs

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**Introduction:** Spices and dried aromatic herbs can be cultured where hygiene conditions might be poorly controlled and products can have high levels of spoilage and pathogenic microorganisms. Since spices and dried herbs are commodities with low water activity they are usually stored at room temperature under dry conditions. Hence they have a shelf-life of 2–3 years.

**Purpose:** Although drying can inhibit microorganism growth, it may not completely inactivate pathogens. Thus the purpose of this study was to investigate survival of pathogens during storage of spices and dried herbs.

**Methods:** We performed a meta-analysis on the available published data to identify the most critical factors that influence survival in spices and dried herbs. Additionally survival of different pathogenic microorganisms was monitored experimentally in powdered paprika under controlled storage conditions.

**Results:** From the meta-analysis we concluded that storage temperature and water activity both play significant roles in survival. Experimental studies which simulated storage conditions showed that different pathogens do not survive to the same extent under the same storage conditions as *Salmonella* spp. had a fifteen times lower inactivation rate than *Listeria monocytogenes*.

**Significance:** Reduction of pathogens during storage of spices and herbs might be limited depending on the type of organism present. Control of the initial levels of microbial contaminants is therefore of importance.

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### 3.13 Microbial distributions in dried oregano batch

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Spices and herbs are non-homogenous matrices which can be naturally contaminated with large numbers of microorganisms, among them pathogenic species and toxigenic moulds. Microorganisms are often heterogeneously distributed in food matrices, which influences the results of sampling. Various sampling strategies may give different probabilities of detection of these microorganisms. In contrast, our knowledge about how microorganisms are distributed in dried powdered food products is relatively scarce. The aim of this study was to investigate the actual contamination levels and the corresponding distributions of bacteria in a powdered spices/dried herbs matrix for *Salmonella* spp., Enterobacteriaceae, sporeformers, *Bacillus cereus* and Total Viable Counts (TVC). For detecting *Salmonella* spp. the Most Probable Number method was used (10 g, 1 g, 0.1 g × 3) with selective media RV and MSRV and confirmation with XLD and BGA. To assess the concentration of Enterobacteriaceae we used selective media of VRBGA and OF confirmation method. Sporeformers concentrations (both aerobic and anaerobic) were investigated with plate counts on PCA. Our results indicated absence of salmonellas and high concentration of TVC (4.5–5.5 log CFU/g) and Enterobacteriaceae (3.5–4.5 log CFU/g). Sporeformers concentrations were at levels ~4.5 log CFU/g. *Bacillus cereus* were present in all tested samples at levels 2.5–3.5 log CFU/g. Results from the distribution fitting using obtained data provided a number of applicable distributions for each category of bacteria, for instance lognormal and log-logistic distributions showed acceptable goodness of fit with TVC at aerobic condition. The analysis of data has extended our knowledge of microbial distributions in powdered matrices and can be helpful for quantitative microbiology in risk assessment of spices and herbs and for evaluating performance of sampling plans.

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### 3.14 Meta-analysis of irradiation data of bacteria in spices and herbs

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**Introduction:** Spices and herbs are natural dried components or mixtures thereof; used in foods for flavouring, seasoning and imparting aroma. Currently in the EU the most consumed spices are pepper, paprika and pimento (allspice), whereas the most consumed herbs are thyme and oregano. Despite their low water activity, which inhibits microbiological growth, spices and dried herbs can be naturally contaminated with large numbers of microorganisms, among them several pathogenic species and toxigenic moulds, for example, *Salmonella* spp., *Escherichia coli*, *Clostridium perfringens*, *Bacillus cereus* and aflatoxigenic *Aspergillus* spp. Spices and herbs are therefore treated for reduction of microbial load.

**Purpose:** The purpose of this study is to identify factors influencing inactivation of pathogenic bacteria by irradiation.

**Methods:** Meta-analysis on the published data available on irradiation was performed and trends were tested for their significance with statistical tests.

**Results:** Inactivation data collected showed high variability in reduction kinetics. Irradiation treatment (gamma or electron beam) and product physical state (whole, ground or powdered) were not found to significantly influence reduction. Gram positive bacteria were significantly more resistant than Gram negative; subsequently spores were significantly more resistant than vegetative cells.

**Significance:** Irradiation may not be able to significantly reduce spores and Gram positive bacteria in spices and dried herbs.

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## 3.15 Microwave treatment of paprika powder in order to maintain colour and improve microbiological status

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### Introduction

Paprika powder is one of the most important food ingredients in the Hungarian cuisine. Its cultivation and usage has centuries old history. Paprika powder products from the two most important traditional growing sites at the southern region (Szeged, Kalocsa) gained permission to use the “hungarikum” label, which is the most respected product label in Hungary, representing the highest of quality.

Traditional paprika powder processing consists of harvesting, drying, decontamination with saturated steam, then milling. Saturated steam decontamination is capable to reduce high amount of microflora, however, it also weakens the colour of the product, which is a very important parameter in terms of quality.

### Aim, materials, methods

Our aim was to evaluate the effect of microwave treatment on paprika powder, while the sample is being kept moved all along. The continuous movement of the samples ensures homogenous treatment temperature, thus avoids darkening and burning. For this process, a specially modified domestic microwave oven was used. A full factorial experimental design was used for 3 parameters: moisture content (20 %, 30 %), treatment temperature (80 °C, 90 °C) and temperature keeping time (0 min, 10 min). Every point was measured once. A centre point (25 % initial moisture content, 87.5 °C treatment temperature, 5 min temperature keeping time) was added to the design, measured by triplicate. Microbiological properties (CFU, mould, yeast, *E. coli*, coliforms), colour (CIELab) and aroma components were measured from the samples. Batch weight was 120 g, original moisture content was 9.24 %. Colour is significantly changes with the change of moisture content, therefore after the treatments, the samples was post-dried to the initial value in terms of comparability. The post-drying (until the samples reached 9.18 % moisture content) was done with vacuum drying at low temperature (30 °C) to avoid further loss of colour and aroma components.

### Results and discussion

According to the results, it can be stated that the most significant factor for microbial reduction was temperature keeping time. Higher values resulted higher CFU reduction. *E. coli* and coliforms were under detection limit at the control sample, but mould and yeast were present, and both of them were almost completely eliminated by microwave treatment. Colour change was barely visible, especially for lower temperatures. Moisture content did not have significant effect on both quality properties.

## 3.16 Identification of biological warfare agents as contaminants in spices and herbs

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Securing the food chains from primary production to consumer-ready food against major deliberate, accidental or natural contaminations is directly related to the safety of food products. In almost every processed food, including ready-to-eat products, spices and herbs are natural products that can be contaminated with several toxins, which can lead to food-borne intoxications. The characterisation of the heterogeneous matrices of spices and herbs and their respective production and supply chains in context with relevant biological hazards and the improvement of the knowledge on biological hazard properties as well as on-site and high throughput diagnostic methods for appropriate detection are main tasks within the EU FP7 project SPICED.

Our research within the SPICED project is mainly focused on the toxins ricin, which is the highly toxic poison from castor beans of *Ricinus communis*, and the enterotoxin type B produced by the Gram-positive bacteria *Staphylococcus aureus* (SEB). Their qualitative and quantitative detection in spices and herbs including the development of sample preparation methods were performed. For this purpose different types of spices and herbs were spiked with the toxins, stored at room temperature and analysed e.g. with immunological based methods. Investigations included also performance of fieldable technologies and methods for the rapid on-site detection. Additionally, decontamination studies for facilities contaminated with toxins were conducted.

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## 3.17 Effects on the structure of the proteins ricin and SEB

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In almost every processed food, including ready-to-eat products, spices and herbs are present. Due to the fact that spices and herbs are natural products they can be contaminated with several toxins, which can lead to food-borne intoxications. Securing the food chains against biological and chemical contaminations is the key issue within the EU FP7 project SPICED. Therefore the characterisation of the heterogeneous and complex matrices of spices and herbs and their respective production and supply chains in context with relevant biological hazards and the improvement of the knowledge on biological hazard properties as well as on-site and high throughput diagnostic methods for appropriate detection are main tasks. Within the SPICED project our institute mainly focusses on the toxins ricin, which is the highly toxic poison Ricinus communis (from castor beans), and the enterotoxin type B (SEB) produced by the Gram-positive bacteria *Staphylococcus aureus* (SEB). For this purpose the structure of both proteins were analysed via high performance liquid chromatography (HPLC). The goal is the availability of an in vitro method for demonstrating measurable effects on the structure of proteins like ricin and SEB after exposing e.g. to physical and chemical influences. A description of the method for structure analysis and the effects resulting from the used influences will be given.

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### 3.18 Examination of spice paprika of different origins

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In order to assess the composition of bioactive ingredients in spice paprika products and to support the safety of the spice product chains, a wide range of compositional examinations were performed on commercial spice paprika samples. Samples from several countries were analysed to find the most important differences in their characteristic properties. Measurements were carried out on spice paprika samples of Hungarian and foreign (Serbian, Spanish, Chinese, Bulgarian, Peruvian) origin. Bioactive components, contaminants, aroma and microbiological properties were analysed, compared and sorted on the base of near infrared spectroscopy.

Overall, 53 paprika samples were collected, with product choice to include different origins and quality. The selected sample group contained spice paprika samples of Hungarian (22 from Szeged and Kalocsa) and foreign (6 Serbian, 7 Spanish, 14 Chinese, 2 Bulgarian and 2 Peruvian) origin.

The Spanish and Peruvian samples were outstanding in total carotenoids content. The ratio of capsanthin diesters to free capsanthins of spice paprika samples are proposed a indicators of origin, supposedly by being related to the climate of the given country. In Hungarian spice paprika samples the concentrations of vitamin C and total tocopherols were high. Aroma components characteristic to the geographical location were detected in each spice paprika sample. The microbiological purity and heavy metal contamination of each sample was found to be appropriate. According to the results of NIR evaluation of spice paprika samples, there occurred some clustering among the samples according the country of origin. Yet, no clear correlation between the examined bioactive components and places of origin has been identified, therefore further studies are needed.

## 3.19 Comparative chemical analysis of environmental and cultivated paprika samples from ecological and conventional paprika crop fields

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Traceability of spice contamination cases is difficult as possible occurrence patterns are very complex. Developing countries such as China, India, Vietnam, Indonesia and the Middle East are among main spice suppliers. In case of spice paprika mycotoxins, illegal dye utilisation, pesticide residues, non-pathogenic microorganisms and heavy metals are the main risk sources.

In field studies on the occurrence of pesticide residues in spice paprika cultivation, three different paprika producers practising intensive cultivation mode and three organic farmers have been involved. Soils of intensive cultivation fields (ICF) and organic cultivation fields (OCF) have been sampled in June and organic fields in July. Two sites of each producer have been sampled, and soil samples have been collected at three different points from two or three depth levels. Thus, altogether 42 soil samples have been collected at six ICFs and 23 soil samples from OCFs. Pesticide residues in soil extracts have been determined by GC-MS. In soils from ICF sampling sites pesticide active ingredients trifluralin, tefluthrin, chlorpyrifos and DDT were detected together with certain decomposition products (DDE, DDD). Harvested paprika samples were collected in September from four ICF and from one OCF as well. Paprika samples have been prepared by modified QuEChERS extraction method and analysed for pesticide residues by GC-MS. No residues could be detected in paprika extracts, even when plants were grown in polluted soil.

In a field model study an intensively treated paprika cultivation site was treated with a mixture of pesticides at three levels. Crop has been treated 1 to 3 times with solutions with pesticide premixes at different concentration (3 levels). Thus, a solution containing 10 g/L Pirimor (50 % pirimicarb), 1.0 g/L Mospilan (20 % acetamiprid), 12.5 mL/L Cyren (480g/L chlorpyrifos) and 7.5 mL/L Danadim Progress (400 g/L dimethoate), as well as its 1:5 and 1:25 dilutions were sprayed on crop stocks. Soils prior to and after the treatments, together with untreated controls, have been sampled. Soil samples were collected twice after first spraying on the first day and followed on the 4$^{th}$ day as well. At the end of the cultivation period, paprika fruits have been harvested. Results well correlated with the doses applied and the numbers of treatment. The higher amounts of pesticides were applied, the higher residue levels in soil and in paprika fruits have been measured.

### 3.20 Ecotoxicological evaluation of wash water in Hungarian spice paprika production

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Spice paprika production is a complete process, from field to the packaged product, where the particularly important aim is to eliminate all contaminant (*i.e.* pesticide residues, microbes) from the fruits. After harvest and post-ripening, the first step is a washing procedure that can remove extensive amount of contaminants from the surface of paprika. Thus, wash water may contain various pesticides and other pollutants.

To assess the efficacy of paprika washing in the technology process, wash water samples were collected from a spice paprika processing plant in Kalocsa, using paprika from conventional cultivation. Water was sampled at the end of the first wash, before water change. Prior to investigating the ecotoxicological effects, pesticide residues and microbiological content of the water were determined. Ecotoxicological evaluation of the water was carried out using three OECD protocols, „*Daphnia* sp. acute immobilisation test" (OECD 202), „Fish acute toxicity test" (OECD 203) and „Fish embryo acute toxicity (FET) test" (OECD 236) on *Danio rerio*. Experiments on *Aedes aegypti* larvae were performed by the corresponding WHO guideline [1] [2]. The *Danio rerio* acute adult test was performed with the original wash water, but for other assays it had to be filtered due to its high contamination of paprika parts.

Analytical analysis determined 0.61 µg/L of chlorpyrifos and microbial contamination of $10^8$ colony forming units/g in the reference wash water sample. The *A. aegypti* test indicated that the test solution was not toxic to L4 stage larvae at a concentration of 100 % (undiluted wash water sample). Results showed that *D. magna* is more sensitive to this mixture: the $LC_{50}$ value was determined to fall between 10 to 20 % of the test solution. First experiments with *D. rerio* adult acute test gave 28 % as $LC_{50}$ value. Nonetheless, the composition of the wash water rapidly changed: mortality determined one week later was 100 % at the same concentration. In the FET test, the $LC_{50}$ value occurred at a concentration of 5.5 %, where half of the population coagulated. Sublethal effects occurred at a concentration line from 3 % to 5 %. Lack of pigmentation, amorphous shape and underdevelopment were the most common as sublethal signs.

Results of our ecotoxicological evaluation emphasise the importance of water-management after the wash procedure in spice paprika processing, since this mixture can cause lethal and sublethal effects on aquatic organisms with a wide range of sensitivity.

[1] http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-2-effects-on-biotic-systems_20745761, accessed 22 April 2016

[2] WHO (2005): Guidelines for laboratory and field testing of mosquito larvicides. pp. 41. http://apps.who.int/iris/bitstream/10665/69101/1/WHO_CDS_WHOPES_GCDPP_2005.13.pdf, accessed 22 April 2016

### 3.21 EU maximum and action levels for potential hazards in culinary herbs and spices

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Although being minor food components, culinary herbs and spices can be contaminated with potential health hazards that are regulated by law. The European Union (EU) food law lays down the general requirements regarding food safety for all member states. For some hazards, maximum levels (MLs) are established for particular food categories. The poster provides an overview on MLs and action levels that are specifically defined for culinary herbs or spices by EU law.

MLs for certain chemical contaminants are laid down by Commission Regulation (EC) No 1881/2006 and amending regulations. Regarding mycotoxins, aflatoxin B1, total aflatoxins (B1 + B2 + G1 + G2), and ochratoxin A are regulated for certain spices. Heavy metal contaminations are limited in leafy vegetables including fresh herbs for cadmium. MLs for polycyclic aromatic hydrocarbons (PAHs) have been laid down for dried herbs and dried spices that are placed on the EU market from 1 April 2016 on, with the exception of cardamom and smoked fruits of *Capsicum* species. Permitted maximum quantities of pesticide residues are regulated by Regulation (EC) No 396/2005 and amending regulations. Specific maximum residue levels are available for fresh herbs and for spices; details are provided in the EU Pesticides database (http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/). For the monitoring of dioxins, furans and dioxin-like polychlorinated biphenyls (PCBs), Commission Recommendation 2013/711/EU defined specific action levels for product categories that include also fresh or dried herbs. The intentional addition of substances to foods for technical purposes is laid down by Regulation (EC) No 1333/2008, Commission Regulation (EU) No 1129/2011, and further amendments. $SO_2$/sulphites are allowed to be added to cinnamon (*Cinnamomum ceylanicum*) and dried ginger at a particular ML. Cellulose and sodium/potassium/calcium salts of fatty acids can be added to dried herbs and spices as required. Food colour(s) are not permitted in herbs/spices, but approved food colours can be added to seasonings. For microbiological hazards, no binding criteria are laid down by EU law specifically for culinary herbs or spices. Recommendations are included in Commission Recommendation 2004/24/EC.

Besides the abovementioned, additional MLs might apply depending on the product. For example, dried vegetables or oilseeds can also cover some spices or can be used for seasonings/condiments. Moreover, fresh spices, such as fresh chili and fresh ginger, are classified as vegetables.

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### 3.22 Microbiological criteria for dried culinary herbs and spices in EU and non-EU member states

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Dried culinary herbs and spices (DCHS) are minor food components with widespread use. If properly dried, they do not support microbial proliferation as a result of their low water activity. But some microorganisms, including pathogens and toxin producers, are able to survive in DCHS. Problems with pathogenic/toxigenic bacteria might particularly arise if microbial contaminated DCHS are added to a foodstuff with a higher water activity in combination with failure in food handling. If subsequent processing steps that inactivate microbial activity are lacking or insufficient, microbes can proliferate in the final product upon improper food storage and/or lead to toxin accumulation. Regarding the microbiological risk related to foods of non-animal origin (FoNAO) in the EU, DCHS in combination with *Salmonella* spp. and *Bacillus* spp. were ranked within the top four food–pathogen combinations (EFSA BIOHAZ, 2013).

An overview is presented on some microbiological criteria that specifically address DCHS and which were found by a literature study and internet research. Compared to food of animal origin, microbiological criteria are, except of few food categories, less defined for FoNAO. In the EU, no microbiological limits specific for DCHS are laid down by the EU food law. However, Commission Recommendation 2004/24/EC provided criteria to assess the biological safety of DCHS on retail and non-retail level within the framework of a coordinated programme for the official food control for 2004. Mandatory microbiological criteria specific for ready-to-eat spices and spice mixtures were found for Luxemburg. Other public standards for spices or DCHS were identified for several non-EU member states including also spice producing countries. In addition to public standards, private standards on microbiological criteria can be available.

In general, the absence of *Salmonella* spp. is the most common criterion found in different multinational and national standards. Moreover, next to total plate count and indicator organisms, microbiological criteria for DCHS often address spore-formers, namely presumptive *Bacillus cereus* and sulphite-reducing clostridia, as well as coagulase-positive staphylococci and mould/yeast fungi. In addition to legal standards, the spice/herb industry and the retail usually use individual buyer–seller agreements, which might be also based on public and private recommendations.

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Reference: EFSA Panel on Biological Hazards (BIOHAZ), 2013. Scientific Opinion on the risk posed by pathogens in food of non-animal origin. Part 1. EFSA Journal 11(1): 3025

### 3.23 European alerting and monitoring data as inputs for the risk assessment of microbiological and chemical hazards in spices and herbs

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**Introduction**: Food chains are susceptible to contaminations from food-borne hazards, including pathogens and chemical contaminants. An assessment of the potential product-hazard combinations can be supported by using multiple data sources.

**Purpose**: The objective of this study was to identify the main trends of food safety hazards in the European spice and herb chain, and then, evaluate how the data sources can be used during each step of a microbiological and a toxicological risk assessment. Thereafter, the possibilities and limitations of the selected data sources for the risk assessment of certain hazards in spices and herbs are examined.

**Methods**: European governmental alerting and monitoring data and legislation were examined and evaluated for particular product-hazard combinations.

**Results**: Pathogenic microorganisms, particularly *Salmonella* spp. and pathogenic *Bacillus* spp., were identified as a potential concern in black pepper and dried herbs, while mycotoxins like aflatoxin (B1) and ochratoxin A were a probable concern in chilies (including chili powder and cayenne), paprika, and nutmeg.

**Significance**: Evaluating multiple, accessible, data sources can support several steps during the risk assessment process as seen for the hazard identification step. Therefore, identifying the potential spice and herb food safety hazards in the chain and how the data can be used for a risk assessment can support risk assessors in compiling a comprehensive risk assessment.

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### 3.24 Risk ranking of chemical hazards in spices and herbs

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**Introduction:** Spices and herbs bring flavour and nutrients to cuisine; however, they may expose consumers to various contaminants that pose a risk to human health.

**Purpose:** To rank the risks of chemical hazards in selected spices and herbs that have the potential for contamination as input for setting up monitoring programs.

**Methods:** A risk ranking toolbox for food and feed related issues (van der Fels-Klerx *et al*. 2015) was used to systematically select a ranking method. The method was applied to rank chemical hazards in paprika/chili, black pepper, nutmeg, basil, thyme, and parsley leaves. The severity and probability of the hazards were scored as low, medium, high, or severe. Literature and data were collected from scientific publications, alerting and (national) monitoring data, and other relevant European Union (EU) reports and databases to determine the scores.

**Results:** A risk matrix approach was selected to rank various chemical hazards including natural contaminants such as mycotoxins and plant toxins, environmental contaminants such as pesticides, dioxins, heavy metals, and polycyclic aromatic hydrocarbons, and deliberate contaminants such as dyes. The risk ranking showed that the following hazards were seen as the riskiest with respect to human health: the mycotoxins aflatoxins and ochratoxin A, the pesticides chlorpyrifos and triazophos, and the dye Sudan I.

**Significance:** A risk matrix provides a transparent risk ranking approach based on available data without the intensive demands required by risk assessments. These results can assist EU initiatives focusing on sampling strategies for monitoring programmes with respect to chemical contaminations in spices and herbs.

Acknowledgements: This research was executed in the framework of the EU project SPICED (Grant Agreement: 312631) with the financial support from the 7$^{th}$ Framework Programme of the EU. Financial contributions from the Dutch Ministry of Economic Affairs are acknowledged.

### 3.25 Pesticide residues in spice paprika in the RASFF system

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Intensive agricultural practice relies on various agrochemicals. Due to the inappropriate application of the pesticides to given commodities, incorrect circumstances of storage and harvest, as well as the illegal use of agrochemicals in commodities, residues of these chemical compounds may occur in agricultural products, therefore, several chemical, physical or biological agents may have potential adverse effects on food safety. The national authorities on food safety in the member states of the EU regularly monitor the agricultural crops and products for compliance with contamination regulations on the basis of official maximum residue levels (MRLs) these organic microcontaminants. Information exchange among member states and to the public is ensured by the Rapid Alert System for Food and Feed (RASFF) of the EU, founded in 1979. Moreover RASFF is the most effective device to report non-compliances of given commodities and food products on the basis of relevant requirements on food safety with real time exchange of information.

In *Capsicum* species, including chili and spice paprika pesticide residues are relatively frequent chemical contaminants. Currently nearly 50 pesticide active ingredients are registered in the EU for paprika cultivation, and these pesticides are dominated by insecticides. Spice paprika consists of a complex plant matrix; therefore, analytical determination of pesticide residues is a complex task, seen in high variability of the analytical results for this spice commodity. In 2014, similarly to previous years, spices and herbs were between the top 10 product categories of reported notifications in RASFF. Numerous non-compliances have been related to spice paprika since 1998, each year. The RASFF Annual Reports collect and publish the current alerts, information notifications and border rejection notifications, with the level of non-compliance contamination found, country of complaint and the exporter country specified. The first declared cases on non-compliance in spice paprika occurred in 1999 reported endosulfan, acephate, methamidophos and cypermethrin, the reporting countries were Finland and Germany, the origin of the export were Spain and Pakistan. Ethion, methamidophos and triazophos represent the most often reported active ingredients. The highest detected level of residue was declared in 2001, when the residue level of ethion reached 12.6 mg/kg concentration in case of imported ground chili in the United Kingdom.

### 3.26 Application of Bayesian networks modelling for the design of a monitoring system for contaminants in spices and herbs

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**Introduction:** The global market provides the consumer with access to a wide variety of spices and herbs. Various chemical, microbial, or physical hazards may occur in each of these spices and herbs. Therefore, these products should be carefully monitored at different points of the spice and herb supply chain.

**Purpose:** This study aimed to determine the hazards-spices/herbs combinations that have an elevated probability of contamination, at each level of the supply chain (suppliers, border inspection points, market, and consumers), using a modelling approach.

**Methods:** A Bayesian Network (BN) Modelling approach was used to determine the probability of contamination of each of the spices and herbs with a certain food safety hazard. Various datasets were used to obtain values for input parameters of the model. These included Rapid Alert System for Food and Feed (RASFF), Dutch national monitoring programme for chemical contaminants in food and feed (KAP), Eurostat, and the EU pesticide database. Part of the data was used for model calibration, and a separate part was used for model validation.

**Results:** The correctness of BN model predictions was higher than 85 %. The results demonstrated that the sampling plan (hazards-spices/herbs to be checked) should depend on the place in the chain at which samples are collected. For example, at EU border inspection, the controller should focus more on curry leaves, chili pepper and curry. The main hazard categories detected at this point are pesticides (41 %), mycotoxins (39 %) and pathogens (17 %). The presented model can aid spice and herb industry and governmental authorities in defining which products and hazards to check at border inspections and along the spices and herbs supply chain.

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### 3.27 Protocol of sampling technologies and corresponding statistics in feed/food lots

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**Introduction:** Because of the variability in both the strategy for collecting samples and sample test results, some contaminated lots may incidentally be classified as acceptable. The choice of the sampling strategy is very important in the development of any sampling plan.

**Purpose:** This study focused on the investigation and evaluation of different sampling strategies and statistics considering the heterogeneity of contaminant distribution in a dry feed/food materials lot. The sampling approaches that were compared include: simple random sampling (SRS), stratified random sampling, (STRS) and systematic sampling (SS).

**Methods:** A Monte Carlo simulation model was developed to compare the different sampling strategies (SRS, STRS, and SS) with respect to their ability to detect a heterogeneously distributed chemical or microbial contamination in a lot of dry feed/food materials, e.g. spices or herbs.

**Results and discussion:** The results showed that in lots with low levels of contamination (1 % and 2 %), the SS strategy is preferred and the number of samples to be taken should be 100 %. At the higher contamination rates (5 % and 10 %), the STRS strategy is preferred and the number of samples to be taken should be higher or equal to 50 %. It is, thus, recommended to define the sampling strategy on the expected contamination rate of the lot.

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### 3.28 Data reporting in the culinary herb/spice chain

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Flux of information relevant for food safety and traceability can be a challenge in the supply chain of dried culinary herbs and/or spices (DCHS). These commodities are in many cases produced outside of the European Union (EU) and production often involves small-scale farmers in less developed countries. Depending on the product, batches consisting of products from different harvests and different farmers are not unusual. To gain an insight into the way data is recorded and reported along the chain, a survey was performed.

Information was provided by 12–15 businesses that produce, process, and/or trade DCHS. Participants were located within and outside the EU; all of the latter exported DCHS to the EU. All these participants were certified to at least one private food safety management scheme, and four to an ISO 9001 quality management system.

Most of the EU participants utilised IT systems for the management of batch numbers, enterprise resource planning (ERP), and/or warehouse management. Among the non-EU participants, implementation of such systems was less frequent. Application of a laboratory information management system (LIMS) and automated data capture were in general rare. The ways and forms of data reporting for DCHS appeared as highly divers, also within the EU. Mostly, information was exchanged as paper document typed with typewriter or computer, or as electronic document that cannot be read out automatically. Some businesses (survey participants and suppliers of those) still used paper documents that were filled in by hand—even for the delivery to the EU. As form for reporting, mostly individual/company-specific forms were used. Standardised forms provided by herb/spice associations were rarely shared. The reported data covered, next to information on product safety characteristics and other parameters that were not surveyed, the most important data for product traceability.

In summary, the survey results indicate the high variability in the way of reporting that can depend on the supplier/next buyer as well as on the product; standardised and/or electronic systems seem to be rare. The extent of data reporting upon import to the EU and within the EU appears as sufficient for tracing one step back and one step forward along the chain; some additional traceability information were frequently reported.

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## Consortium

This unique consortium is composed of eleven experienced institutions from seven European countries and includes partners from industry, academia and food authorities.

**Project partners**

1. Bundesinstitut für Risikobewertung (Federal Institute for Risk Assessment), Germany
2. Austrian Agency for Health and Food Safety, Austria
3. Institute of Food Safety, Animal Health and Environment, Latvia
4. DLO Foundation – RIKILT, the Netherlands
5. FUCHS Gewürze GmbH, Germany
6. National Agricultural Research and Innovation Centre, Hungary
7. RTD Services, Austria
8. University of Limerick, Ireland
9. National Agricultural and Food Centre, Slovakia
10. Bundeswehr Research Institute for Protective Technologies and NBC-Protection, Germany
11. Wageningen University, the Netherlands

**Integrated stakeholders**

12. European Spice Association, Germany
13. Fachverband der Gewürzindustrie, Germany
14. Van Hees GmbH, Germany
15. Kräuter Mix GmbH, Germany

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