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Antimicrobial activities of spices and herbs against *Salmonella* Oranienburg

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ABSTRACT

The detection of *Salmonella* spp. within dried spices and herbs is challenging due to their potent antimicrobial activity. In the present study nine condiments were investigated whereof oregano and cinnamon led to a complete inhibition of *Salmonella* Oranienburg at a 1:10 dilution in buffered peptone water, while towards allspice and thyme an adaptation was apparent. At a next step, a tenacity study was set up where the survival of *S.* Oranienburg in dry condiment samples was followed qualitatively and quantitatively during storage at 25 °C for 365 days. Generally, a higher susceptibility of *S.* Oranienburg to spices than herbs was determined. Furthermore, to the best of the author's knowledge, this is the first study presenting a significant antimicrobial effect of paprika/chilli against *Salmonella*. Nevertheless, *S.* Oranienburg was able to persist during storage in several condiment samples with a low reduction of $\log_{10}$ <1.5 colony-forming units g^{-1}. Thus, pointing to the outstanding ability of *S.* Oranienburg to survive dry storage conditions even spiked to condiments, known for their high antimicrobial activity.

1. Introduction

Since the 1970s the worldwide production of spices and herbs is significantly increasing due to a higher demand of the population on flavorful and exotic seasonings (FAO, 2016). Spices and herbs are ready-to-eat foods without further need for cooking or processing to fulfill their criteria from a microbiological point of view, and thus are considered as critical foods (EC, 2005). Although several guidance documents are available to meet the required standards regarding spice production, processing and use (ASTA, 2011; Codex Alimentarius, 1995; ESA, 2015), the food category herbs and spices is among those with the highest number of notifications according to the European Rapid Alert System for Food and Feed (RASFF, 2016). More than 500 notifications are directed to >80 different *Salmonella* serovars in >60 herbs and spices from 2003 to date. Van Doren, Kleinmeier, Hammack, and Westerman (2013) concluded from a comprehensive study in the fiscal years 2007–2009 that *Salmonella* in spices and herbs imported to the United States showed a prevalence of 6.6%, twice as much as the mean value out of all other foods. Considering underreporting, Scallan et al. (2011) estimated that approximately 1.0 million illnesses per year occur in the United States due to foodborne *Salmonella*. In Europe, almost 90,000 confirmed cases and 1049 outbreaks of salmonellosis were reported for 2014, representing 23.4 cases per 100,000 population (EFSA & ECDC, 2015b). Moreover, the matrix-pathogen combination of *Salmonella* in spices and herbs is among the most relevant according to the European Food Safety Authority (EFSA, 2013) and Da Silva Felício et al. (2015), however, the relative abundance in Europe was <1% in the years 2013 and 2014 (EFSA & ECDC, 2015a, 2015b). On the other hand, the minimal infectious dose for susceptible persons is only 1–10 colony-forming units (CFUs) g^{-1} product or even below (Hammack, 2012; Lehmacher, Bockemühl, & Aleksic, 1995), while for healthy people it is considered to be several logs higher (Bell & Kyriakides, 2002; WHO & FAO, 2002). After ingestion and passage of the stomach, nontyphoidal *Salmonella* adhere to epithelial cells of the intestine, followed by an invasion into the cells and expression of virulence factors. Diarrhea and vomiting, and also often fever, can be detected 6–72 h after ingestion (Hammack, 2012; Stephan, Lehner, Zweifel, & Hächler, 2014).

Salmonella do not produce spores as clostridia or bacilli, however, they are well prepared with their versatile tool set to persist harsh conditions as the storage in low moisture environments and foods (Dubois-Brissonnet, Naïtali, Mafu, & Briandet, 2011; Finn, Condell, McClure, Amézquita, & Fanning, 2013; Gruzdev, Pinto, &

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Sela, 2011; Spector & Kenyon, 2012). Although they cannot grow under these circumstances (Beuchat et al., 2013), they might persist months and up to several years (Finn et al., 2013). Additionally, *Salmonella* are generally heterogeneously distributed among several subsamples within these foods, which is also emphasized by RASFF notifications from 2003 to date where in only 6% of the cases all subsamples (3, 4 or 5) were positive, while in 45% only one subsample (out of 3, 4, 5 or 10) was positively confirmed (RASFF, 2016).

In the present study *Salmonella* Oranienburg was investigated with respect to its susceptibility towards antimicrobial effects of nine spices and herbs during 1:10 (w/w) enrichments. Furthermore, different carrier materials for spiking dry condiments were investigated with regard to the recovery of *S.* Oranienburg. Afterwards, the dry condiments were artificially spiked in a tenacity study where the *Salmonella* population was followed qualitatively and quantitatively during storage at 25 °C for 365 days.

2. Material and methods

2.1. Salmonella strain

A *Salmonella* strain was used, which was previously isolated from a native ground cumin sample during routine analysis in the laboratory of the Austrian Agency for Health and Food Safety Ltd. (AGES) where 1 of 5 subsamples (each 25 g) was positive. The serological profile was determined by the AGES national reference laboratory for *Salmonella* with the somatic (O) antigen 6, 7, 14 (group O:7), and the flagellar (H) phase 1 antigen m, t and phase 2 antigen [z_{57}]. Thus, the present strain was confirmed as *Salmonella enterica* subsp. *enterica* serotype Oranienburg (*S.* Oranienburg) (Grimont & Weill, 2007). Due to the fact that this strain was able to persist in a dried spice for a prolonged period, it was expected to be predestined for the present study.

2.2. Matrices: spices and herbs

Nine spices and herbs were obtained from FUCHS Gewürze (Germany), namely allspice, basil, black pepper, cinnamon, nutmeg, oregano, paprika/chilli, parsley and thyme. All samples were dried and then grounded to particle sizes of <1 mm. The spices and herbs were steam treated to improve the microbiological status of the condiments, except oregano and basil. The sample batches were specifically prepared for the EU project SPICED (Grant Agreement: 312631).

2.3. Status zero of the spices and herbs

The status zero of the matrices was determined in triplicates according to the following standards: ISO 4833-1:2013 for total mesophilic aerobic count, ISO 7932:2005 for presumptive *Bacillus cereus*, ISO 21527:2008 for moulds, ISO 7937:2004 for *Clostridium perfringens*, ISO 6888-2:1999/Amd.1:2003 for coagulase-positive staphylococci (*Staphylococcus aureus* and other species) and ISO 16649-2:2001 for *Escherichia coli*, while the detection of *Salmonella* spp. is described below.

2.4. Salmonella detection

The detection of *Salmonella* spp. was performed according to ISO 6579:2002-Cor.1:2004-Amd.1:2007 with a dilution of 1:10 (w/w) with buffered peptone water (BPW) (Merck) for a non-selective enrichment, shortly mentioned as 'ISO 6579' (Fig. 1). Alternatively, a 1:20 (w/w) dilution and supplementation of the BPW with 0.5% (w/v) K_2SO_3 (Sigma-Aldrich) to reduce potential antimicrobial

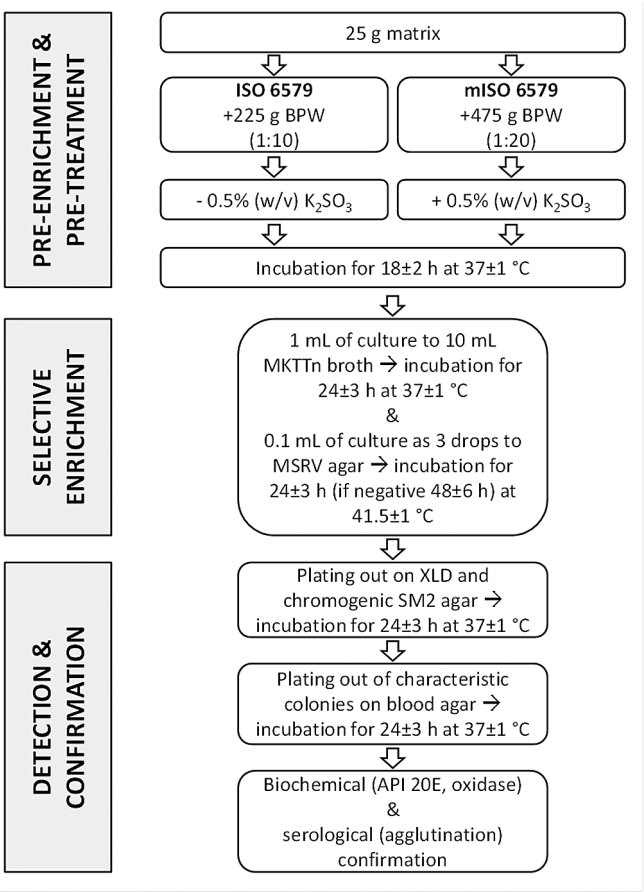

Fig. 1. Methodological scheme. **BPW**, buffered peptone water; **ISO 6579**, strictly sticking to the ISO; **mISO 6579**, modified ISO with 1:20 (w/w) dilution with BPW supplemented with 0.5% (w/v) K_2SO_3; **MKTTn**, Muller-Kauffmann Tetrathionate-Novobiocin broth; **MSRV**, Modified Semi-solid Rappaport-Vassiliadis agar; **XLD**, xylose lysine deoxycholate agar.

effects of the matrices, as for example endogenous propyl disulfides (D'Aoust, 2009), was done according to ISO 6887-4:2003-Cor.1:2004-Amd.1:2011 and referred to as 'm(odified)ISO 6579'. Presumptive *Salmonella* colonies were streaked on xylose lysine deoxycholate (XLD) agar according to ISO 6579 as well as chromID *Salmonella* (SM2) agar (bioMérieux) as a second selective agar. If a CFU value was ≥20, a weighed arithmetic mean with the CFU of the prior dilution was calculated. Additionally, qualitative analyses according to the above mentioned ISO norms were done with Muller-Kauffmann Tetrathionate-Novobiocin (MKTTn) broth and a Modified Semi-solid Rappaport-Vassiliadis (MSRV) agar (bioMérieux).

2.5. Antimicrobial effects of condiments at 1:10 (w/w) with BPW

At the commencement of the study it was necessary to screen the nine matrices for potential antimicrobial effects in comparison to the control without matrix. For that, an experiment was set up where $\log_{10}$ 6.0 CFU of *S.* Oranienburg were added to 25 g of the respective matrix, subsequently diluted 1:10 (w/w) with BPW and incubated at 37 ± 1 °C. Samples were withdrawn for qualitative and quantitative detection of *S.* Oranienburg directly after inoculation (time 0 h) and 2, 4, 6, 12, 24, 48 or 72 h afterwards. At least three dilutions were each streaked on XLD agar in triplicates.