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Investigation of regional differences of the dominant microflora of spice paprika by molecular methods

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ABSTRACT

As microbial contamination of spices comes primarily from the soil, and soil microbiota are dependent on climate, geography, agriculture, etc a qualitative focus on the dominant microflora of spices instead of the quantitative determination of commonly studied spoilage and pathogenic microflora may give a better insight into the geological origin of the sample. The aim of the present study was to identify using molecular microbiological methods the dominant bacteria of spice paprika produced at different countries and to look for species characteristic of spices from the given regions. According to our data, no differentiation could be made among spice paprika samples of different geographical origin using the total bacterial count or extent of mould contamination. However, when the dominant microflora is examined, bacterial species could be identified in the spice paprika samples characteristic of a particular climate. According to our study, the presence of *Bacillus mycoides* and *Bacillus licheniformis* was characteristic of the microflora of spice paprika grown in Central Europe; *Bacillus safensis* could be detected in all four paprika samples examined from the tropical monsoon climate; the species common to all three samples of the tropical climate group were *Bacillus amyloliquefaciens* subsp. *plantarum* and subsp. *amyloliquefaciens*, while *Bacillus mojavensis* was detected as being characteristic of Spanish origin for paprika. No common species were found in the paprika samples originating from China.

1. Introduction

Spice paprika is a condiment commodity made from air-dried fruit of the chilli pepper family of the species *Capsicum annuum*. Although paprika is often associated with Hungarian cuisine, the chillies from which it is made are native to the New World, introduced to the Old World from the Americas. Originating in Central Mexico it was brought to Spain in the 16th century. The trade of paprika expanded from Iberia to Africa and Asia, and ultimately reached Central Europe through the Balkans, then under Ottoman rule (Andrews, 1995, p. 186). Central European paprika had a typically hot taste until the 1920s when a Szeged breeder found a variety that produced a sweet tasting fruit, and then grafted it onto other plants (Sasvari, 2005, p. 280).

Paprika is commonly used to add flavour and colour to various ethnic dishes, but is also added to many foods, such as baked goods, drinks, meat, soup, ice cream, candy as well as condiment mixes, to provide flavour and red colouring. Thus, the consumer may have a large intake of paprika in the diet without realizing it.

Outbreaks of diseases of food origin in both developing and developed countries have heightened consumer awareness regarding food quality and safety issues concerning aspects of geographical origin, agricultural practices and accurate labelling of food products. One of the most important safety issues of spice paprika are toxins produced by fungi colonizing paprika berries during growth and also during storage after harvest. Mycotoxins are climate-dependent, plant- and storage-associated problems, also influenced by non-infectious factors (e.g. bioavailability of (micro) nutrients, insect damage, and attacks by other pests), that are in turn driven by climatic conditions. Climate represents the key agro-ecosystem driving force for fungal colonization and mycotoxin production (Magan, Hope, Cairns, & Aldred, 2003). Thus, the

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place of origin of spice paprika is a good indicator of mycotoxin hazards.

The presence of mycotoxins is not the only health hazards to consumers. Nowhere, at the present time, are pesticides more valued than in developing countries, particularly those in tropical regions seeking to enter the global economy by providing off-season fresh fruit and vegetables to countries in more temperate climates. Such developing nations are becoming important 'breadbaskets' for the world, capable of growing two or even three crops a year. However, these goals cannot be achieved without the increased use of pesticides, agents not as extensively used in traditional agricultural practices (Forget, , Goodman, , & deVilliers, 1993). Banned toxic agricultural chemicals, even where they are not used anymore, may still be present in the soil from past use. Farms and food processors may be situated in heavily industrialized regions, for example in China, where water, air, and soil can be contaminated by industrial effluents and emissions. Contamination from human and animal waste also contributes to poor water quality, partly because most rural areas in developing countries lack adequate sewage systems. It is common practice to let livestock and poultry roam freely and to spread livestock and poultry waste on fields used for crop growth (Gale & Buzby, 2009).

There have been attempts to determine the geological origin of food products. For wines, geographic identification based on chemical composition and isotopic analysis is very advanced. Several analytical methodologies (liquid and gas chromatography, mass spectrometry, nuclear magnetic resonance spectroscopy, electronic tongue and nose) and the use of multivariate analysis tools have been successfully used for this purpose. In wine, chemical analysis provides the basis for territorial information (Acevedo, Jimenez, Maldonado, Dominguez, & Narvaez, 2007; Cynkar, Dambergs, Smith, & Cozzolino, 2010; Fabani et al., 2010). Analysis of trace elements and ratios of stable isotopes, that depend on factors, such as environmental contamination, agricultural practices, climate, and vinification processes, have been shown to be a valuable tool to discriminate wines according to their region of origin (Suhaj & Korenovská, 2005). According to Soós et al. (2014), the electronic tongue was suitable to analyse white wine samples made from different grape types which originated from different wine regions of Hungary.

For spice paprika, a method based on the measurement of colour was proposed to differentiate samples from different geographical origin by Palacios-Morillo, Jurado, Alcázar, and Pablos (2016). $\delta^{18}O$ fruit water and corresponding source water and also compound specific stable hydrogen isotope data were analysed and correlated to the regional GNIP (Global Network of Isotopes in Precipitation) values by de Rijke et al. (2016) for geographic origin discrimination of bell peppers.

Many spices are grown and harvested in poor sanitary conditions in areas of high heat and humidity. Such conditions permit potential microbiological contamination. Numerous studies have indicated high microbial loads in spices and herbs as reviewed by McKee (1994). Those determinations were focussed on total counts, mould contamination, pathogenic bacteria, and, high level of microbial contamination was reported from samples originating around the world. Besides the quantitative determination of the microbial contamination, only a few studies have carried identification of the microflora of spices, and they are mostly done using selective culturing for pathogenic species, such as *Bacillus cereus*, *Salmonella* spp, *Clostridium perfringens*, *Escherichia coli*, etc (Antai, 1988; Banerjee & Sarkar, 2004; Sagoo et al., 2009).

Microbial contamination of spices comes primarily from the soil, and the soil microbiota are dependent on climate, geography, agriculture, etc, qualitative determination of the dominant microflora of spices instead of the quantitative determination of commonly studied spoilage and pathogenic microflora could give a better idea of the geological origin of the sample. Therefore, the aim of this current work was to identify using molecular microbiological methods the dominant bacteria of spice paprika produced in different countries and to investigate for the occurrence of species that may be characteristic to spices from a particular region.

2. Materials and methods

2.1. Paprika samples

Seventy-one spice paprika samples from 10 countries (Hungary 29, China 15, Serbia 7, Spain 7, India 3, Bulgaria 2, Brazil 1, Peru 1, Kenya 1, Thailand 1, and unknown origin 4) were received either from Fuchs Gewürze GmbH as part of the EU-FP7 SPICED project for the microbial contamination studies, or, obtained from the Hungarian market. For place of origin studies spice paprika and chilli samples were collected from the Hungarian market (HU1, SE, SP, IN1, IN2, IN3, TH, CH1, CH2, KE), received from Fuchs Gewürze GmbH were (BR, PE, CH3), and that obtained from a Hungarian small producer (HU2) (Table 1). The samples collected could be grouped by their climate of origin as shown in Fig. 1.

2.2. Enumeration

Serial dilutions were carried out in peptone-water (peptone 1 g/l, NaCl 9 g/l) from 10 g of paprika. Mesophilic aerobic total count was determined in PCA (Plate Count Agar – Merck, Budapest, Hungary) incubated at 30 °C for 3–5 days. Mould and yeast counts were determined on Chloramphenicol Glucose Agar (Biolab, Budapest, Hungary) incubated for 5 days at room temperature.

Table 1
Spice paprika samples used in the place of origin studies.

ID	Name of the product	Place of origin	State	Package size
HU1	Paprika Powder Mild	Hungary	powder	50 g
HU2	paprika from private producer	Hungary	powder	–
SE	Paprika Mild	Serbia	powder	100 g
SP	Paprika Powder Mild	Spain	powder	100 g
BR	–	Brazil	powder	–
PE	–	Peru	powder	–
IN1	Red Dry Chilli Hot	India	shredded	40 g
IN2	Bird Eye Chilli Extra Hot	India	shredded	50 g
IN3	Chilli Powder Extra Hot	India	powder	100 g
TH	Kim-Chi Paprika	Thailand	shredded	50 g
CH1	Kashmiri Chilli	China	powder	50 g
CH2	Bio Chilli Powder	China	powder	50 g
CH3	–	China	powder	–
KE	Dried Chilli Pepper	Kenya	whole	100 g