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Fungi in spices and mycotoxigenic potential of some *Aspergilli* isolated

Marcelo Valle Garcia, Gilson Parussolo, Camila Brombilla Moro, Angélica Olivier Bernardi, Marina Venturini Copetti*

Food Science and Technology Graduate Program, Federal University of Santa Maria - UFSM, CEP 97105-900, Santa Maria, RS, Brazil

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ABSTRACT

The aim of this study was to identify fungal species present in 200 samples of rosemary, fennel, cinnamon, clove, pepperoni, black and white pepper and oregano and evaluate the mycotoxigenic potential of the some *Aspergilli* isolated. Clove, black and white peppers were analyzed by direct plating. For rosemary, cinnamon, fennel, pepperoni pepper and oregano samples were used spread plate. Mycotoxigenic capacity was verified by the agar plug method. With the exception of clove, all the spices showed high fungal contamination, especially by *Aspergillus* sp., *Penicillium* sp. and *Cladosporium* sp. Frequency of toxigenic *Aspergillus* spp. was intense in white and black peppers, with presence of *Aspergillus flavus* (up to 32%), *Aspergillus nomius* (up to 12%), *Aspergillus parasiticus* (up to 4%), *Aspergillus niger* complex (up to 52%), *Aspergillus ochraceus* (up 12%) and *Aspergillus carbonarius* (up to 4%). 14,2% of *A. flavus* isolated from black pepper were aflatoxins producers. In the white pepper, 66.7% of *A. flavus* isolates and 100% of *A. nomius* were aflatoxigenic. Oregano showed the highest number of *A. niger* complex isolates (49), however, only 2.04% produced ochratoxin A. This study showed a huge fungal presence in spices, which could compromise the sensorial quality of these products and represent a hazard for consumers.

1. Introduction

Spices are considered non-perishable products under normal storage conditions and some of them possess antimicrobial and antioxidant properties allowing the food preservation (Ascenção and Filho, 2013). Even then, some of these products can be an important source of microbial contamination when added as ingredient during the food preparation (Ruiz-Moyano et al., 2009; Da Silva et al., 2012; El Maghubi et al., 2013; Teixeira-Loyola et al., 2014).

The spices come from various parts of plants such as bark, buds, flowers, fruits, leaves, rhizomes and stigmas. The majority come from tropical countries and is used to flavoring, coloring or preserving food and beverages (FAO, 2005). In tropical climate is common the occurrence of high temperature, humidity and rainfall rates. These climate parameters are suitable for intense microbial growth, especially of fungi. This group of microorganism is widely distributed in soil, organic matter and water and can easily contaminate the spices (Pitt and Hocking, 2009). In later stages, intrinsic and extrinsic parameters can allow fungal multiplication, especially the water content of the substrate. So, the dehydration step is critical and can confer high load of viable microbial cells to spices if carried out slowly, besides it can cause a reduction in the product quality, especially sensory, and safety (FAO, 2005).

Considering the high importance of aroma and flavor for most spices, the modification of sensory characteristics by fungi through the production of volatiles compounds is critical and could depreciate their market value. There is also an additional concern regarding the possibility of mycotoxin formation during the fungal growth, representing a hazard to public health (IARC, 1993; Pitt and Hocking, 2009).

In order to minimize these problems of microbiological origin, the *Codex Alimentarius* created a specific committee for spices and culinary herbs, where the main objective was the establishment of international standards and development of a code of practice for the safe production of these products. Associated with this, the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) conducted an international call for data on any microbiological risk associated with these foods (FAO, 2013). So, this study aimed to evaluate the fungal contamination in rosemary, cinnamon, clove, fennel, oregano, white, black and pepperoni pepper as well as the mycotoxigenic potential of selected

* Corresponding author.
E-mail address: mvc@smail.ufsm.br (M.V. Copetti).

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Aspergillus species.

2. Methods

2.1. Samples

A total of 200 samples of spices were analyzed, corresponding to 25 lots of rosemary leafs (*Rosmarinus officinalis* L.), cinnamon powder (*Cinnamomum cassia* Ness.), clove flowers (*Syzygium aromaticum* L.), fennel seeds (*Pimpinella anisum* L.), white and black peppercorns (*Piper nigrum* L.), pepperoni pepper powder (*Capsicum baccatum* L.) and oregano leafs (*Origanum vulgare* L.). The samples were marketed in the city of Santa Maria, RS, Brazil. The samples were bought separately in portions of 100 g and packed in polyethylene bags. They were carried to the laboratory and analyzed as soon as possible (in the same week), kept at room temperature, as in the market. The samples presented no indication of their origin in the label.

2.2. Water activity analysis (a_w)

The water activity was determined in triplicate directly in a water activity meter (Aqualab Series 4 TE, USA), at 25 °C ± 1. The amount required to measure the a_w followed the manufacturer's instructions, enough to fulfill half of the recipient (~2 g).

2.3. Fungal contamination of spices

Spreading plate method was chosen for analyses of powdery samples of rosemary, cinnamon, fennel, pepperoni pepper and oregano. Briefly, 25 g of each spice was weighed and aseptically added to 225 ml of peptone water (0.1%). After homogenization, serial dilutions were carried out with subsequent inoculation in three plates of Dichloran Glycerol Agar 18% with chloramphenicol (DG18) and incubated at 25 °C for seven days. Results were expressed in colony forming units per gram of product (CFU/g) (Pitt and Hocking, 2009).

Particulate samples of clove, black and white pepper were analyzed by direct plating method with a previous disinfection in sodium hypochlorite solution (0.4%) for one minute. Then, 33 particles were arranged in three Petri dishes containing DG18. The plates were incubated at 25 °C for seven days and the results expressed as percentage of infected particles, as Pitt and Hocking (2009).

2.4. Identification of filamentous fungi

The colonies were isolated on Czapek Yeast Extract Agar (CYA) and subsequently identified according to recommendations for each fungal genus. The identification of *Aspergillus* spp. followed Klich and Pitt (1988) and Frisvad et al. (2004) and identification of species of *Penicillium*, the keys proposed by Pitt (2000) and Frisvad and Samson (2004). The fungi were inoculated in different culture media and after the period of cultivation had the diameters of the colonies measured and the macro and microscopic characteristics of each fungi was observed.

2.5. Mycotoxigenic capacity test

Fungi identified as potential producers of aflatoxins and ochratoxin A were inoculated on Yeast Extract Sucrose agar (Samson et al., 2004) for 7 days at 25 °C and then the agar plug technique (Filtenborg et al., 1983) was used to evaluate the capability of isolates to produce mycotoxin. Fungal extracts taken as plugs with a cork borer were placed on TLC plates, developed in a toluene: ethyl acetate: formic acid 90%: chloroform (7:5:2:5, v/v/v/v) mobile phase, and visualized under UV light at 365 nm. Aflatoxins B1, B2, G1 and G2 and ochratoxin A standards were used for comparison.

2.6. Data analysis

Data were presented and expressed as average of fungal infection between samples of each spice, frequency of occurrence (FO) (% number of samples with occurrence of a genus (or species)/total number of samples) and variation of infection (VI) (% of minimum and maximum fungal infection observed in the samples).

3. Results and discussion

The analyzed spices showed average value of water activity (a_w) of 0.67. Clove samples showed the highest average aw values (0.78, ranging from 0.57 to 0.82) (data not shown) and fennels (0.62) the lowest (Table 1).

In general, the levels of a_w in the samples were low, suggesting adequate drying and storage. The reduction in the a_w of foods is applied by the industry for the maintenance of the quality of a food product, promoting better utilization of raw materials, increasing the shelf-life, as well as acting for microbial control (Troller, 1991).

With the exception of clove, that showed no fungal contamination in all 25 analyzed samples (<10 CFU/g), the spices showed high fungal counts. No correlation was observed between aw value in a sample and the fungal loud on it. However it influenced the group of fungi present in the samples. Fungi xerophilic or moderately xerophilic were dominant.

A total of 22 genera and 51 different fungal species were isolated from the samples (Table 1). Overall, the xerophilic species *Aspergillus ruber, Aspergillus chevalieri, Aspergillus montevidensis, Aspergillus pseudoglaucus* and *Aspergillus penicillioides* were the most common fungi isolated from the spices; as well, the mycotoxigenic species *Aspergillus flavus* and *Aspergillus niger* complex. Several species of *Penicillium* and other genera such as *Cladosporium* and dematiaceous fungi were also commonly present in spice samples.

Our mycological results support the observations made by Hocking (1981), which sets that due to tropical origin and methods applied during the production, spices are often contaminated with xerophilic fungi in very high scores. The fungi of the genus *Aspergillus, Penicillium* and *Aspergillus* with sexual status *Eurotium* are often the dominant biota of dried, whole or ground spices (Pitt and Hocking, 2009). The same authors also pointed out that the fungi of the genus *Aspergillus* sexually state *Eurotium* are widely distributed in low water activity food. Fungi of these genera have also been reported as responsible for the production of volatile compounds in grains and in stored foods. The production of these compounds implicates in changes of organoleptic characteristics, especially in spices, since these products are mainly appreciated by its sensorial attributes (Borjesson, 1993; Cao et al., 2017).

The average of fungal contamination among spices ranged from 1.73×10^3 CFU/g in pepperoni pepper and $6{,}02 \times 10^4$ CFU/g in rosemary and 76,3% and 77% regarding average infection in white and black pepper, respectively. White pepper samples showed fungal contamination ranging from 18.2% to 100% of peppercorns and *A. chevalieri* was the predominant species found. *A. niger* complex was the second most frequent species isolated from this spice. *A. chevalieri* was present in 64% of black pepper samples, followed by *A. niger* complex (52%) and *Aspergillus tamarii* (36%), respectively.

Pepperoni pepper had the lowest average score among the evaluated fungal spices, between <10 and 8×10^3 CFU/g. The xerophilic species *A. montevidensis*, *A. ruber* and *Wallemia sebi* were the most isolated fungi. Oregano samples showed counts ranging