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Risk Assessment of Mycotoxins and Predictive Mycology in Sri Lankan Spices: Chilli and Pepper

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Abstract

This research contributes to a better understanding of the mycotoxin problem associated with two important spices in world trade;, chilli and pepper by a multidisciplinary approach including analytical chemistry, risk assessment, food chemistry and predictive mycology. More specifically, this work provides important insights in mycotoxin contamination of these spices and associated risks in Sri Lanka. Firstly, a simple extraction method based on the QuEChERS approach was developed and successfully validated for the simultaneous determination of multiple mycotoxins using an advanced chromatographic technique, LC-MS/MS. The method was applied on complex spices for quantitative screening of seventeen mycotoxins. In addition to the classical aflatoxins and ochratoxin A, the spices were also found to be contaminated with several other toxicologically significant mycotoxins. Chilli samples (87%) were more frequently contaminated with mycotoxins than peppers (65%). Subsequently, the mycotoxins screening results and the collected consumption data were integrated in a quantitative risk assessment study. The results showed that AFB1 exposure via chilli consumption is of a public health concern in Sri Lanka, pepper is of lesser extent a risk due to the lower consumption. The toxigenic mould characterization in black peppers showed that Aspergillus flavus and/or Aspergillus parasiticus were the predominant moulds (73%) found, with considerable contamination (60%) of Penicillium spp. and A. niger. Furthermore, predictive mould growth models on peppercorns were developed at three temperatures and seven water activity levels for both A. flavus and A. parasiticus isolates. Based on the research, suitable storage conditions for black peppercorns were suggested and the way forward in managing the risk towards mycotoxins posed by the consumption of these two spices in Sri Lanka.

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1. Introduction

Sri Lanka, "The Pearl of Indian Ocean" in South Asia is also known as "Spice Island", where highly priced spices are produced, consumed and exported. Spices are well known for imparting flavour, colour, aroma in diverse cuisines and for therapeutic properties. However, their quality is often compromised. As a developing country Sri Lanka has its own limitations in producing high quality spices for local consumption and in compling with trade regulations enforced by the importing countries. Moreover, as a tropical nation the prevailing climatic conditions, while supporting the spice crop development could also be highly favorable for mould infestation and mycotoxin contamination in the field or during post-harvest practices. Mycotoxins are toxic secondary metabolites produced by diverse[1]. filamentous fungi. Like many other foods, spices could also be contaminated with moulds and mycotoxins affecting their safety and quality. Hence, this research work is carried out in order to identify the actual situation in moulds and mycotoxin contamination in spices and to perform a quantitative risk assessment of mycotoxins in Sri Lanka, given the limited information on the mycotoxin issues therein. In this study, chilli (Capsicum annum L.) and pepper (Piper nigrum L.) were selected since these two are important spices of world trade and consumption.

2. Methodology

First, a reliable and rapid method was developed based on a QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) extraction procedure for the determination of multiple high-performance liquid chromatography mycotoxins in spices, chilli, black and white peppers.[2] Tandem mass spectrometry (HPLC-MS/MS) was used for the quantification and confirmation of chemically diverse mycotoxins. Mycotoxins were extracted from the hydrated spices using acidified acetonitrile, followed by partitioning with NaCl and anhydrous MgSO4 excluding the use of dispersive-solid phase extraction. Electrospray ionization at positive mode was applied to simultaneously detect all the mycotoxins in a single run time of 20 min. Multiple reaction monitoring mode, choosing at least two abundant fragment ions per analyte was applied. Recoveries (75 to 117%) were in accordance with the performance criteria required by the European Commission (EC, 401/2006). The limit of quantification (LOQ) ranged from 2.3 to 146 µg/kg. The method LOQ meets the maximum levels (MLs) of the two regulated mycotoxins, aflatoxins and ochratoxin A (OTA) in spices hence, it could be used for the purpose of enforcement of the EU MLs. The validated method was finally applied to screen mycotoxins in chilli and pepper samples collected from Sri Lanka. Chilli is mainly imported from India, while the pepper is cultivated in Sri Lanka itself.

After the screening and measuring the status of the contamination of available spices, statistical analysis was performed to identify significant differences between region of sampling, form of spices (e.g. flakes, whole, powder, etc). Also a quantitative risk assessment (deterministic and probabilistic) of mycotoxins due to the consumption of chilli and black pepper in Sri Lanka was conducted. A food frequency questionnaire was administered in order to collect the data on consumption of spices by households in the Northern and Southern region (n=249).

In a next step, the growth and mycotoxin production of an A. parasiticus and three A flavus isolates in whole black peppercorns using a full factorial design with seven water activity (aw) (0.826-0.984) levels and three temperatures (22, 30 and 37°C) were used to develop predictive models. Growth rates and lag phases were estimated using linear regression. Several secondary kinetic models were assessed for their ability to describe the radial growth rate as a function of individual and combined effects of aw and temperature.*Tables*

All tables should be numbered with Arabic numerals. Every table should have a caption. Headings should be placed above tables, left justified. Only horizontal lines should be used within a table, to distinguish the column