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Screening of moulds and mycotoxins in tomatoes, bell peppers, onions, soft red fruits and derived tomato products

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ABSTRACT

International standards and European legislation are available for well established mycotoxins such as aflatoxins (AF), ochratoxin A (OTA) and trichothecenes in a variety of dried plant products. However, information of mycotoxins in fresh fruit and vegetable produce and derived products is limited. A semi-quantitative screening method was developed to screen for six mycotoxins (alternariol (AOH), alternariol monomethyl ether (AME), OTA and fumonisin B1, B2 and B3 (FB)) relevant in different matrices (tomatoes, bell peppers, onions and soft red fruits). On tomatoes, onions and soft red fruits, *Alternaria* spp. and their associated mycotoxins were detected. Derived tomato products were also screened and six out of 173 samples and four out of 173 samples were positive for AOH and AME, respectively. Moreover, 11/11 derived tomato products, containing AOH or AME, were positive for tenuazonic acid (TeA) as well. A dietary exposure assessment was performed for TeA with Belgian consumption data, and the obtained mean value (4230 ng/kg bw/day) was higher than the threshold of toxicological concern (TTC) value of 1500 ng/kg bw/day set by EFSA. This study demonstrates the necessity for further mycotoxin research in the fresh produce chain in order to guarantee the safety and health of the consumers.

1. Introduction

Scientific literature, risk assessment studies and legislation are available on well established mycotoxins such as aflatoxins (AF), ochratoxin A (OTA) and trichothecenes in different dried plant products (e.g. cereals, maize) (van Egmond, Schothorst, & Jonker, 2007; EU regulation 1881/2006). However, information for mycotoxins in fresh produce or derived processed products (e.g. pasteurized or canned products) is limited. Only the presence of patulin in apple products and OTA in dried fruits, grapes and wine is well documented and also regulated in Europe (Baert, De Meulenaer, Kamala, Kasase, & Devlieghere, 2006; Blesa, Soriano, Molto, & Manes, 2006; EU regulation 1881/2006; Lombaert et al., 2004). In order to estimate the prevalence of emerging mycotoxins on fresh produce and derived products a number of mycotoxins and matrices were selected in this research. This selection was made based on (1) a literature research, (2) expert opinion and knowledge and (3) their frequent use in derived food products and thus the theoretical risk of exposure for consumers to the possible presence of mycotoxins in derived food products. A first product that meets these criteria are tomatoes. Tomatoes are not only eaten fresh, they are also processed into a variety of products, such as sauces, ketchup, pulp, paste, juices and dried tomatoes. There is a large consumption of these products by the population, which means that the possible presence of mycotoxins could have a significant impact on public health (Mariutti & Soares, 2009; Muhammad, Shehu, & Amusa, 2004).

Abbreviations: AF, aflatoxin; AME, alternariol monomethyl ether; AOH, alternariol; DON, deoxynivalenol; DRBC, dichloran Rose Bengal Chloramphenicol; FB, fumonisin; HPLC, high-performance liquid chromatography; LB, lower bound; LC-TOF-MS, liquid chromatography-Time Of Flight-Mass Spectrometry; LOD, limit of detection; LOQ, limit of quantification; MEA, malt extract agar; OTA, ochratoxin A; TeA, tenuazonic acid; UB, upper bound.

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Tomatoes are highly susceptible to fungal infestation due to their soft epidermis (Moss, 1984). The most common fungi that infect the tomato plant and the tomatoes are *Alternaria* species. The occurrence of *Alternaria* mycotoxins in tomato products has been reported in Argentina (Somma et al., 2011; Terminiello, Patriarca, Pose, & Fernandez Pinto, 2006), Switzerland (Noser, Schneider, Rother, & Schmutz, 2011), Brazil (da Motta & Soares, 2001) and Germany (Ackermann et al., 2011; Asam, Liu, Konitzer, & Rychlik, 2011). Tenuazonic acid (TeA) is reported to occur in higher concentration compared to other *Alternaria* mycotoxins such as alternariol (AOH) and alternariol monomethyl ether (AME). AOH and AME, on the other hand, are possibly genotoxic (EFSA, 2011a).

Next to tomatoes, also sweet bell peppers can be associated with mycotoxins. Recently, internal fruit rot of sweet bell peppers has become a problem in several European countries (i.e. Belgium, the Netherlands, and UK) and Canada. Several *Fusarium* species are suspected of causing this phenomenon (Monbaliu et al., 2009a, 2009b).

Next to tomatoes and sweet bell peppers, also onions can be of concern. Since the phytopathogenic *Fusarium proliferatum* (which can produce fumonisin B1 (FB1)) was associated with bulb rot on onion plants (Dissanayake, Tanaka, & Ito, 2009; Dugan, Hellier, & Lupien, 2003; Stankovic, Levic, Petrovic, Logrieco, & Moretti, 2007) and a study in Serbia showed that there is also a potential mycotoxin risk in onion plants which are contaminated with *F. proliferatum* (Stankovic et al., 2007), also onions can be of concern. At this moment, there are no data available of contaminated onions with mycotoxins.

A last group of fresh produce potentially at risk are soft red fruits (i.e. small berries such as strawberries, blueberries, blackberries, red currants and raspberries). They have a soft skin and are thus susceptible for small lesions which allows the growth of spoilage fungi both in the pre- and post-harvest stage. So far, little information is known on the presence of mycotoxins in soft red fruits or derived products. A study in Austria analyzed seven berry juices for patulin but none of the samples were contaminated. In Sweden 42 samples of blueberry soup and purees were tested for patulin but none of them were contaminated (EU report on tasks for scientific cooperation, 2002).

In this study, tomatoes, sweet bell peppers, onions and soft red fruits were screened for emerging mycotoxins (AOH, AME, TeA) and more established mycotoxins (OTA and fumonisins (FB1, FB2 and FB3)) in order to have a first insight in the potential risks for public health related to the presence of mycotoxins in those fresh produce and their derived products. Considering the derived products, the goal was not to focus on dried products, since it is expected that the mycotoxin pattern would be different in such products. Next to the screening for mycotoxins, also the moulds present on those fresh produce were isolated and identified. The presence of patulin in these products was evaluated separately and will be reported later.

2. Materials and methods

2.1. Chemicals and reagents

LC-MS grade water, acetic acid and formic acid were supplied by Fluka (Sigma–Aldrich, Bornem, Belgium). LC-MS grade methanol, ethyl acetate and acetonitrile were supplied by VWR (Leuven, Belgium).

Standards of AOH, AME, OTA, FB1, FB2 and FB3 were supplied by Sigma–Aldrich (Bornem, Belgium). The standards were dissolved in methanol, except for FB1 and FB3, FB1 was dissolved in water/acetonitrile 1:1(v:v) and FB3 was bought as a solution (water/acetonitrile, 1(v):1(v)). The solutions were dried under nitrogen and kept at −28 °C.

2.2. Sample preparation

2.2.1. Sample collection

Mouldy samples of fresh tomatoes (n = 161), bell peppers (n = 47), onions (n = 61) and soft red fruits (n = 50) were collected on several markets in different countries (i.e. Belgium, Spain, Egypt, Brazil, India and South-Africa). Mouldy samples were kept at room temperature for some more days. After they were completely moulded, the samples were stored at −28 °C. Derived products of tomatoes such as ketchups, concentrates, pulp, dried tomatoes and juices were collected on local markets in different countries (i.e. Belgium, Spain, Egypt, Brazil and South-Africa). A total of 144 samples of derived tomato products were screened.

2.2.2. Spiking of blank samples

The matrices (fresh tomatoes, bell peppers, onions and soft red fruits with no visible moulds contamination) were homogenized by use of a blender. Before extraction the blank samples were spiked with a working solution at different levels. The samples were mixed during 1 h using a rotary shaker (Labinco, Breda, The Netherlands) to allow equilibration with the matrix.

2.2.3. Extraction

Two ml of extraction solvent (acetonitrile/ethyl acetate/formic acid 60:39:1) was added to 1 g of the homogenized sample. After vortexing, it was mixed using a rotary shaker (Labinco, Breda, The Netherlands). The mixture was centrifuged during 10 min at 9000 rpm (Sigma 4k15, Buckinghamshire, England). A volume of 750 μl of extract was transferred into eppendorf tubes and dried under a gentle stream of nitrogen and reconstituted in 750 μl of methanol. After vortexing and sonication, the sample was filtered through a 0.45 μm syringe filter and analyzed by LC-TOF-MS.

2.3. Instrumental parameters

High resolution liquid chromatographic separation was achieved on an Ultimate 3000 RSLC system (Dionex, Amsterdam, The Netherlands), consisting of a vacuum degasser, binary pump, cooled auto sampler, column oven (30 °C), and equipped with a Zorbax SB-C8 column (Agilent Technologies, Diegem, Belgium). Mobile phase A consisted of water/methanol/acetic acid 95:4.9:0.1 and mobile phase B of methanol/water/acetic acid 97:2.9:0.1. The gradient was a linear increase from 30 to 100% of mobile phase B in 23 min and back to 30% mobile phase A at a flow rate of 0.2 ml/min. One run took 29 min. Injection volume was 20 μl.

The RSLC system was interfaced split less to a time-of-flight mass spectrometer (microTOF II, Bruker Daltonics, Bremen, Germany) equipped with an orthogonal electrospray ionization (ESI) source operating in both positive and negative mode. At the beginning of each run, the MS was calibrated with a sodium-acetate calibrant solution (0,1% acetic acid, 1% 1 M NaOH in a water/isopropanol mixture (50/50)).

2.4. Validation

The recovery of the extraction method was validated by spiking blank samples of mixed tomatoes, bell peppers, onions, soft red fruits (blueberry, redcurrant, strawberry and blackberry) with a known amount of mixed standards (0–200 ng/g). This procedure was done in duplicate and used 6 different concentrations (0–20–50–100–150 and 200 ng/g). The total recovery (TR), signal suppression-enhancement (SSE) and extraction efficiency (EE) were calculated as described by Sulyok, Berthiller, Krska, and Schuhmacher (2006): TR (%) = 100 × slope c/slope a, where a is the slope of spiked pure solvents and c is the slope of the calibration