Review

Sterigmatocystin: A mycotoxin to be seriously considered

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ARTICLE INFO

Keywords:
Foods
Mycotoxins
Sterigmatocystin
Analytical methods

ABSTRACT

Sterigmatocystin is a carcinogenic compound that affects several species of crops and several species of experimental animals. The sterigmatocystin biosynthetic pathway is the best known and most studied. The International Agency for Research on Cancer classifies sterigmatocystin in the Group 2B. Three groups of analytical methods to determine sterigmatocystin in food can be found: chromatographic, ELISA immunoassays and chemical sensors. In addition, sterigmatocystin is a precursor of aflatoxin B_1 in those cases where cereals and/or food are contaminated with fungi capable of producing aflatoxins. Chemical structures of sterigmatocystin and aflatoxin B_1 are similar. These mycotoxins are pathogens of animals and cereals, producing a major economic impact on biotechnology and agricultural and food industries.

This review summarizes different aspects related to sterigmatocystin such as its biosynthesis, toxicological studies and analytical methods for its determination.

1. Introduction

Mycotoxins (from the Greek words "*mykes*": fungus and "*toxicum*": poison) which mean fungus and poison are toxic secondary metabolites of low molecular weight produced by filamentous fungi. They contaminate various agricultural commodities either before harvest or under post-harvest conditions and cause a toxic response when are ingested, inhaled or absorbed through the skin by humans and animals. These diseases are known as "mycotoxicosis" (Soriano del Castillo, 2007; Wagacha and Muthomi, 2008; Flores-Flores et al., 2015).

During January–June 2004, an aflatoxicosis outbreak in eastern Kenya resulted in 317 cases and 125 deaths. Another smaller outbreak was reported in 2005 and 2006, with 30 cases and 9 deaths, respectively (Azziz-Baumgartner et al., 2005).

The International Agency for Research on Cancer (IARC) reported that 500 million of the poorest people in sub-Saharan Africa, Latin America and Asia are exposed to mycotoxins at levels that substantially increase mortality and morbidity (Neme and Mohammed, 2017).

The exposure of humans to mycotoxins occurs directly through ingestion of contaminated agricultural products such as cereals, fruits, etc. (primary mycotoxicosis) or indirectly through the consumption of products of animal origin obtained from animals which were fed with contaminated material such as milk, eggs, etc. (secondary mycotoxicosis) (Fig. 1) (Flores-Flores et al., 2015).

Currently, more than 300 mycotoxins have been identified and scientific attention was mainly focused on those mycotoxins that have proven carcinogenic and/or toxic (Ji et al., 2016), some of them are considered to be of world-wide importance (Miller and Trenholm, 1994) (Table 1).

In this work, we present different aspects related to mycotoxin sterigmotocystin (STE) such as its biosynthesis, toxicological studies, and analytical methods for its determination. STE is a mycotoxin that must be taken into account for its ability to have a significant impact on human and animal health.

2. Sterigmatocystin

STE was isolated by the first time in 1954 from *Aspergillus versicolor* cultures (Soriano del Castillo, 2007). STE is a precursor of aflatoxin B_1 (AFB_1) in those cases where cereals and/or food are contaminated with fungi capable of producing aflatoxins.

Chemical structures of STE and AFB_1 are similar (Fig. 2). AFB_1 is the most potent carcinogenic mycotoxin known (IARC, 2006; Miller and Trenholm, 1994; Soriano del Castillo, 2007).
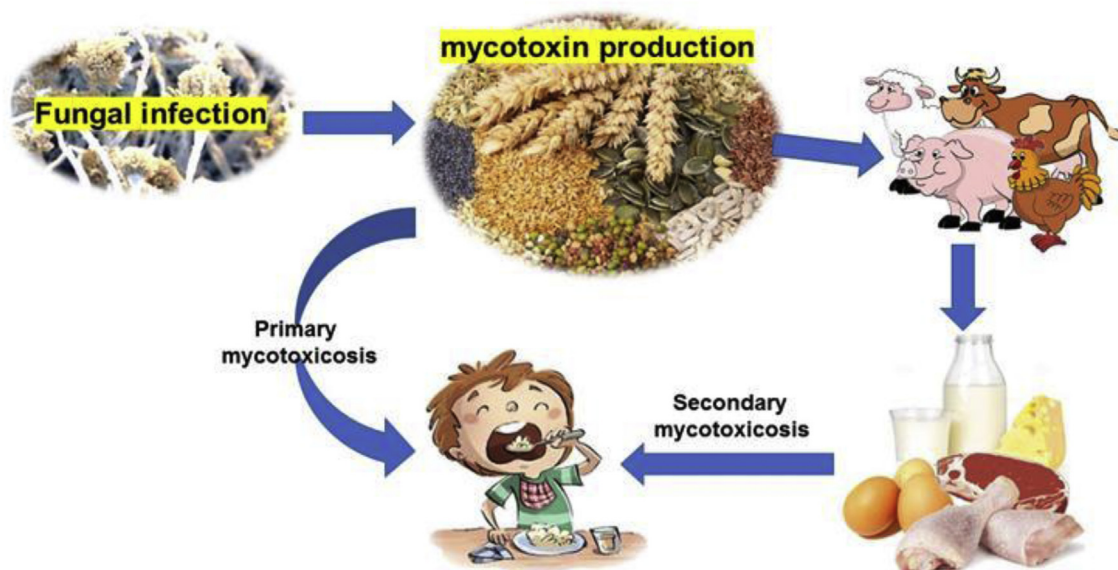
Fig. 1. Primary and secondary mycotoxicosis.

Table 1
Moulds and mycotoxins of world-wide importance.

Mold species	Mycotoxins produced
Aspergillus parasiticus	Aflatoxins B_1, B_2, G_1, G_2
Aspergillus flavus	Aflatoxins B_1, B_2
Fusarium sporotrichioides	T-2 toxin
Fusarium graminearum	Deoxynivalenol Zearalenone
Fusarium moniliforme	Fumonisin B_1
Penicillium verrucosum	Ochratoxin A
Aspergillus ochraceus	Ochratoxin A

STE (IUPAC name: (3aR,12cS)-8-hydroxy-6-methoxy-3a, 12c-di-hydro-7H-furo [3′,2′:4,5] furo[2,3-c] xanthen-7-one) consists in a xanthone nucleus bond to a bifuranic structure. It has a hydroxyl group and a methoxy group. It has a molecular weight of 324.29 g/mol, a melting point of 246 °C and it crystallize as pale-yellow needles (Veršilovskis and De Saeger, 2010). STE is soluble in methanol, ethanol, acetonitrile (ACN), benzene and chloroform (Septien et al., 1993).

STE shows three absorption bands at 235, 249 and 329 nm in ethanolic solutions (Davies et al., 1960). In ACN solutions, STE shows a change in the position of spectral bands (bathochromic shift), with λ = 240, 282 and 321 nm (Díaz Nieto, 2017). In addition, the position of absorption bands are λ = 250, 285 and 328 nm in phosphate buffer solution at pH < 11, while the absorption band positions are λ = 250, 272, 320 and 385 nm in solutions of pH > 12 (Díaz Nieto, 2017).
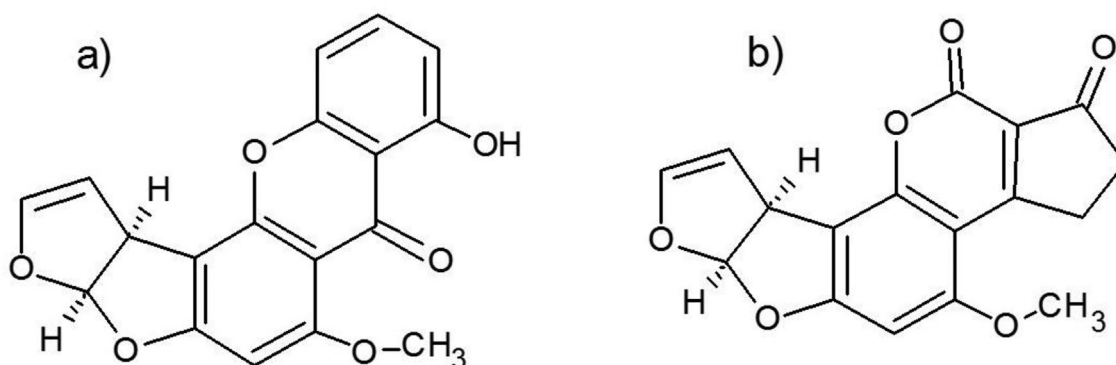
Table 2 shows absorption maxima for different xanthones and STE in ethanolic and ACN solutions. The wavelengths of STE absorption maxima are different to those of xanthone. This is due to the functional groups present in STE chemical structure (phenol, o-methyl and bifuranic ring). In addition, the position of STE absorption bands depends on the interaction of STE with the solvent molecules as it is shown in Table 2 (Wade et al., 2004).

The STE apparent acid dissociation constant, calculated in phosphate buffer solutions, was 8.13×10^{-13} (Díaz Nieto, 2017).

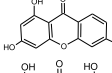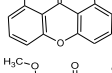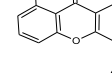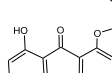STE fluoresces a brick-red color on silica gel TLC plates whereas related structurally compounds fluoresce blue using the same excitation wavelength (Maness et al., 1976).

STE is electrochemically reduced in ACN + 0.1 M tetra-butylammonium perchlorate at glassy carbon (GC) electrodes (Díaz Nieto et al., 2016). Two reduction peaks centered at - 1.77 and - 2.33 V vs. Ag/AgCl were found. The overall electrode process was diffusion controlled; the diffusion coefficient was 3.1×10^{-5} cm^2 s^{-1}. An irreversible dimerization reaction is coupled at the initial electron transfer reaction at the first reduction peak. The proposed mechanism is shown in eqs. (1)–(4). STE here appears as STEH, where H refers to the proton of the phenolic group (Fig. 2).

Fig. 2. Chemical structure of a) sterigmatocystin and b) aflatoxin B_1.

Table 2
Wavelengths of absorption maxima for different xanthones. * Molar absorptivities in parentheses.

Molecular structures	Wavelengths of absorption maxima*	Solutions	References
	239 (39000), 261 (12600), 287 (4200), 337 (6350)	Ethanol	Scott, 1964.
	237 (36000), 251 (22400), 337 (12300)		
	229 (56250), 252 (7940), 334 (1990), 380 (800)		
	236 (40740), 309 (16982)		Davies et al., 1960
	235 (30902), 249 (33884), 329 (16218)		
	240 (37200), 282, 321 (16200)	Acetonitrile	Díaz Nieto, 2017
	250, 285, 328	Buffer phosphate pH < 11	
	250, 272, 320, 385	Buffer phosphate pH > 13	

$$2 \, (\text{STEH} + e^- \rightleftharpoons \text{STEH}^{\cdot -}) \tag{1}$$

$$\text{STEH}^{\cdot -} + \text{STEH}^{\cdot -} \rightleftharpoons {}^- \text{STEH-STEH}^- \tag{2}$$

$$^-\text{STEH-STEH}^- + 2 \, \text{STEH} \rightleftharpoons \text{STEH}_2\text{-STEH}_2 + 2 \, \text{STE}^- \tag{3}$$

$$4 \, \text{STEH} + 2 \, e^- \rightleftharpoons \text{STEH}_2\text{-STEH}_2 + 2 \, \text{STE}^- \tag{4}$$
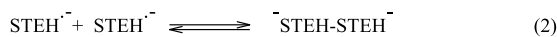
2.1. Biosynthesis and distribution of STE in filamentous fungi

The biosynthetic pathway of STE is the most well-known and studied. This mycotoxin is biosynthesized through the acetate-malonate pathway (Fig. 3a) (Davies et al., 1960; Wilkinson et al., 2004; Yu et al., 2004). Moreover, the molecular characterization of the genes involved in the biosynthetic pathway is well known in literature (Yu et al., 2004). Fig. 3b shows the AFB1 biosynthetic pathway.

Wilkinson et al. (2004) found that the biosynthesis of STE is related to the conidial stage of the fungus, so that STE could contribute to improve the survival of the fungus.

STE can be produced by fungal species phylogenetically and phenotypically different such as *Aschersonia, Aspergillus, Bipolaris, Botryotrichum, Chaetomium, Emericellai, Eurotium, Farrowia, Fusarium, Humicola, Moelleriella, Monocillium* and *Podospora* (Rank et al., 2011).

Slot and Rokas (2011) found that the STE gene group may has been transferred horizontally between phylogenetically unrelated species, such as *Aspergillus* and Podospora.

STE was isolated by the first time from *Aspergillus versicolor* cultures as it was previously mentioned, and it is the source more used to produce STE. Jurjević et al. (2013) studied the ability to produce STE by *Aspergillus* section *versicolor* in two liquid media, Czapek w/20% Sucrose Broth and Yeast Extract Broth grown in the dark during 1 week at 25 °C. Eleven of thirteen species of *Aspergillus versicolor* produced STE. The species isolated on indoor air (*A. creber, A. cvjetkovicii, A. jensenii* and *A. protuberus*) and two species isolated on fruits (*A. amoenus* and *A. fructus*) have the ability to produce STE. *A. versicolor, A. fructus,* and *A. jensenii* produced STE in 10–100 times greater amounts than the other species in the section and represent the greatest threats of STE

contamination.

The *Nidulantes* species, *A. croceus* (isolated in the air and in the sediments collected in the Cave of the Treasury of Andalusia, Spain) and *A. askiburgiensis* (isolated from the sediments collected in the Cave Na Pomezi, Czech Republic) can produce STE (Hubka et al., 2016). The pH is one of the most important external factors to control STE biosynthesis by *A. nidulans*. The STE production increased at alkaline pH (Delgado-Virgen and Guzman-de-Peña, 2009). In addition, regulatory aspects related to the carbon source in STE biosynthetic pathway in *A. nidulans* was studied. Kinetic data revealed that if the carbon source is D-glucose, STE is formed only after the sugar is depleted from the medium, whereas if the source of carbon is lactose, STE appears when the most of the carbon source is still available. This suggests that the formation of STE may be mediated by a mechanism of carbon catabolites, or induced by low specific growth rates attainable in lactose (Németh et al., 2016).

Moreover, STE can also be produced by *A. flavus, A. flavus* var. *parvisclerotigenus, A. parasiticus, A. toxicarius, A. nomius, A. pseudotamarii, A. zhaoqingensis, A. bombycis* and from the ascomycete genus Petromyces (*Aspergillus*, section Flavi), *E. astellata* and *E. venezuelensis* from the ascomycete genus *Emericella* (*Aspergillus*, section Nidulantes) and *A. ochraceoroseus*. These latter fungi produce STE as a precursor of AFB$_1$ (Fig. 3b). In these fungi, STE does not accumulate in large amount because of it is consumed to produce AFB$_1$ (Sweeney and Dobson, 1999; Yu et al., 2004; Frisvad et al., 2005; Rank et al., 2011).

2.2. Toxicological information

It has been found that STE induces tumors in several species of animals, such as mouse, rats, monkeys and fish, after of oral, intraperitoneal, subcutaneous and dermal applications. The malignant tumors include hepatocellular carcinomas, hemangiosarcomas of the liver and pulmonary adenomas (EFSA, 2013; Pfeiffer et al., 2014). A hemangiosarcomas is a type of malignant tumor that originates in the cells that line the blood vessels, while an adenoma is a benign epithelial tumor whose internal structure is like that of a gland.

STE was associated with acute clinical symptoms, bloody diarrhea and death in cattle that ate foods containing the fungus *A. versicolor* (Vesonder and Horn, 1985).

STE causes tumors in the skin of experimental animals after 70 days of cutaneous application (Purchase and Van der Watt, 1973). The mutagenic activity of STE is several times higher than that induced by aromatic hydrocarbons (Tang and Friedman, 1977).

Fushimi et al. (2014) studied the quantification of STE in urine of ruminant cattle that had eaten food contaminated with this mycotoxin. Urine had blood when animals ate contaminated food. After the suspension of contaminated food, STE disappeared from the urine after two weeks.

Cultures of mammalian cells exposed to STE have irregular processes in the nucleus: inhibition of mitosis, inhibition of timidine and uridine catchment, and stimulation of DNA repair synthesis (Engelbrecht and Altenkirk, 1972; Kunimoto et al., 1974; Stich and Laishes, 1975; Terao et al., 1975). STE has also been shown to inhibit RNA synthesis in rat liver (Nel and Pretorius, 1970).

Tian et al. (1995) conducted studies in Chinese patients with liver cancer and cancer of the gastric tissues. The DNA-STE adduct was detected in specimens of cancerous tissues and/or precancerous tissues. STE was detected in urine and plasma of patients with documented hepatocellular carcinoma (Cao et al., 2018).

Zouaoui et al. (2016) investigated the cytotoxicity of individual and combined mycotoxins such as STE, beauvericin (BEA) and patulin (PAT). The cytotoxicity on immortalized ovarian cells (CHO-K1) was also evaluated. After 24, 48 and 72 h, the IC50 values were 2.9 μM for PAT and ranged from 10.7 to 2.2 μM and from 25.0 to 12.5 μM for BEA and STE, respectively. These authors proposed that the co-occurrence of low concentrations of mycotoxins in food may increase their toxic
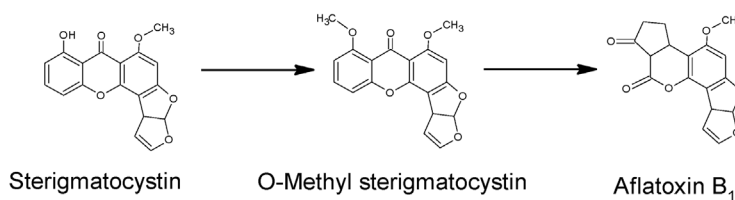
a)
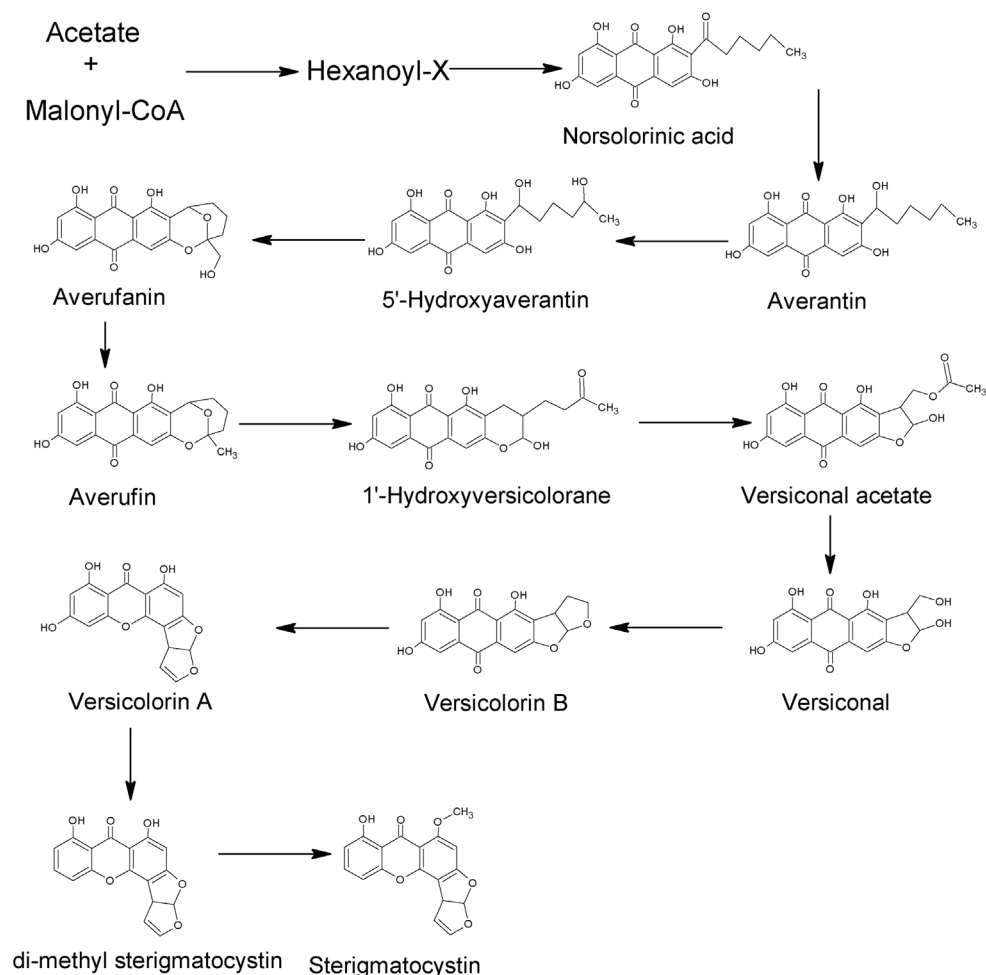
b)

Fig. 3. a) Sterigmatocystin and b) aflatoxin B_1 biosynthetic pathways.

effects. IC50 is the half maximal inhibitory concentration. It is a measure of the effectiveness of a substance in inhibiting a specific biological or biochemical function.

STE exerts genotoxic effects on HepG2 cells, most likely through oxidative stress and lysosomal leakage. IC50 value for STE on HepG2 cells after 24 h of exposure was 3 μM (Gao et al., 2015). HepG2 cells are similar in many functional respects to liver cells. These cells are considered as an ideal cell line when detecting hepatotoxicity and genotoxicity and oxidative stress occurs when the balance between the production and scavenging of reactive oxygen species that can induce lipid peroxidation, DNA fragmentation, and protein oxidation, is interrupted (Nencini et al., 2007). Liu et al. (2014) reported an IC50 of 7.3 μM for STE on HepG2 and 3.7 f μM for human lung adenocarcinoma

(A549) cell line. These authors also studied the hepatocarcinogenic property of both AFB$_1$ and STE in the human hepatoma HepG2. Due to the additive nature of AFB$_1$ and STE to cytotoxicity endpoints, AFB$_1$ and STE might additively promote the apoptosis of HepG2 cells. Moreover, STE was more cytotoxic (Neuro-a2; IC50 = 40.1 μM) towards neuronal cells than other cell lines (Bünger et al., 2004). Apoptosis is a specialized process of cell death that is a part of the normal development of organs and tissue maintenance, but may also occur as a response to various environmental stimuli, indicating toxicity.

STE could induce adenocarcinoma of lung in mice in vivo and DNA damage, cell cycle arrest in a human immortalized bronchial epithelial cell line (BEAS–2 B cells) and a human lung cancer cell line (A549 cells) in vitro. Besides, STE could induce G2 arrest (cell cycle arrest in G2

Table 3
Effects of STE on animals or cells.

Animals or cells	Effects	References
Animals (Mouse, rats, monkeys, fish and cattle)	- Hepatocellular carcinomas - Hemangiosarcomas liver - Pulmonary adenomas - Adenocarcinoma - Inhibit RNA synthesis - Tumor in skin - Bloody diarrhea - Death	Cui et al., 2017; EFSA, 2013; Nel and Pretorius, 1970; Pfeiffer et al., 2014; Purchase and Van der Watt, 1973; Vesonder and Horn, 1985
Cultures of mammalian cells	- Inibition of mitosis - Inhibition of timidine and uridine catchment. - Simulation DNA repair synthesis - DNA-STE adduct synthesis. - Cell cycle arrest in G2 phase	Engelbrecht and Altenkirk, 1972; Essigmann et al., 1979; Kunimoto et al., 1974; Pfeiffer et al., 2014; Stich and Laishes, 1975; Terao et al., 1975; Tian et al., 1995.
Inmortalized ovarian cells (CHO-K1)	- Oxidative stress - Lysosomal leakage	Zouaoui et al., 2016
Hep G2 cells	- Genotoxic effect - Apoptosis	Gao et al., 2015; Liu et al., 2014
Neuronal cell (Neuro_a2)	- Apoptosis	Bunger et al., 2004
Human inmortalized bronchial epitelial cell line (BEAS-2B cells) and human lung Cancer cell line (AS49 cell)	-Cell cycle arrest - Induce DNA double-strand breaks - Affected key proteins involved in cell cycle regulation to trigger genomic instability	Huang et al., 2014

phase) in several other cells. Cell cycle arrest may be one of the common toxic effects of STE. The inducing apoptosis may be a common effect of STE in different cells in vitro (Cui et al., 2017).

STE could induce DNA double-strand breaks in a human immortalized bronchial epithelial cell line (BEAS-2B cells) and a human lung cancer cell line (A549 cells). STE induced DNA damage and affected key proteins involved in cell cycle regulation to trigger genomic instability, which may be a potential mechanism underlying the developmental basis of lung carcinogenesis (Huang et al., 2014).

Table 3 shows a summary of STE's toxicological information.

In the literature is found two different STE mutagenicity mechanisms (Fig. 4). The first was proposed by Essigman in 1979 (Essigmann et al., 1979; Gopalakrishnan et al., 1992). The mutagenicity mechanism is related to the formation of the epoxy group, which is very reactive (1 in Fig. 4). This reactive species can be covalently bound to DNA by generating the STE-N7-guanine adducts. The other mechanism was proposed by Pfeiffer et al. (2014). They suggested hydroxylation of the aromatic ring generating a catechol (2 in Fig. 4). This catechol can react with DNA. It was found that in the liver microsomes of humans and rats the catechol was mainly formed, while the epoxide was formed in small amounts. This suggests that the epoxide pathway is less than the catechol pathway (Pfeiffer et al., 2014).

Despite evidence of genotoxicity and carcinogenicity, only limited database o tumorigenicity is available for dose-response evaluation. With all evidence presented, the IARC classifies STE in the Group 2B (possible human carcinogen) (IARC, 2006).

No country has legislation related to STE levels permitted in food. However, some countries set STE maximum levels allowed in some food. Thus, Czech Republic and Slovakia allow a level of 50 ppb for rice,
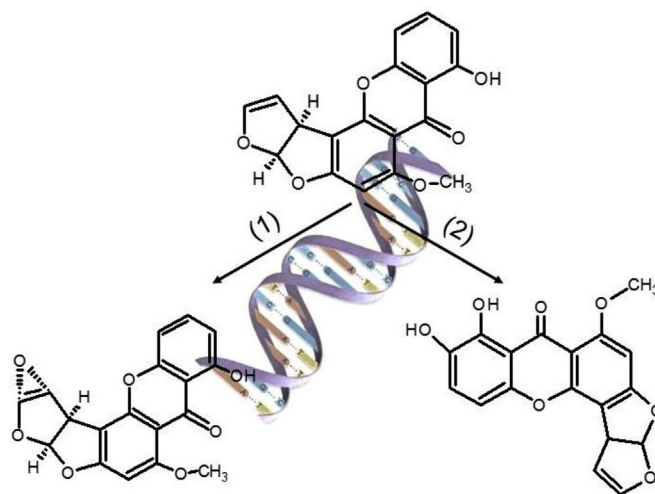

Fig. 4. Two different STE mutagenicity mechanism. 1) Formation of epoxy group, and 2) formation of catechol.

vegetables, potatoes, flour, chicken, meat and milk, and of 20 ppb for other foods. The Department of Health of California (USA) proposes a LD50 of $8\,\mu g\,kg^{-1}$ of body weight for an adult of 70 Kg (EFSA, 2013), being LD50 the amount of a toxic substance that kills 50% of a group of test animals (Soriano del Castillo, 2007).

From the available data about hemangiosarcomas in liver of male rats, a STE dose of $160\,\mu g\,kg^{-1}$ of corporal weight by day was calculated for a response of 10% additional risk (BMD10). The BMD10 is defined as the dose that causes a determined exchange of 10% of response.

2.3. In what substrates can STE be found?

The wider and more discontinuous distribution of this toxin raises an important issue in food safety. The natural presence in foods seems to be rare, but only a limited number of studies have been carried out.

STE was found in cheese contaminated with *A. versicolor* (Scott, 1989). Northolt et al. (1980) reported about the STE occurrence in the cheese maturation in cheese cave from The Netherlands. Cheeses contaminated with STE come of eight warehouses and it is found in all aged categories of cheese (2–8 month), in a concentration range from 5 to $600\,\mu g\,kg^{-1}$. STE can be found in cheese contaminated for a period of 3 months at different temperatures (from -18 to $16\,°C$) (van Egmond, 1983). Lund et al. (1995) studied the production of STE from *A. versicolor* isolated from cheese and air from maturation cave. *A. versicolor* is frequently present in cheese, while aflatoxin-producing fungi such as *A. flavus* and *A. parasiticus* are rarely present in this food (Northolt et al., 1980).

The STE occurrence, distribution and stability in Roumy cheese has also been studied (Abd Alla et al., 1996). A 35% of samples of Roumy cheese were contaminated with STE (concentration range $10–63\,\mu g\,kg^{-1}$). Aged cheeses (over 6 months) inhibited the production of STE.

As cheeses can easily be contaminated with fungi both during the process of their ripening in warehouses, during storage, after cutting and slicing the cheese in the stores or at home, there is a high probability that the cheese will be contaminated with STE.

STE also was found in different spices and cereals, in varying amounts depending on food (Fig. 5).

The first report of STE in spices was in India. STE was found in fennel sample ($142\,\mu g\,kg^{-1}$) and black pepper (105 and $125\,\mu g\,kg^{-1}$) (Saxena and Mehrotra, 1989). In Egypt, STE was detected in red pepper ($10–23\,\mu g\,kg^{-1}$), caraway seeds ($14–18\,\mu g\,kg^{-1}$), cumin and marjoram (El-Kady et al., 1995). In cumin and marjoram, STE was co-produced
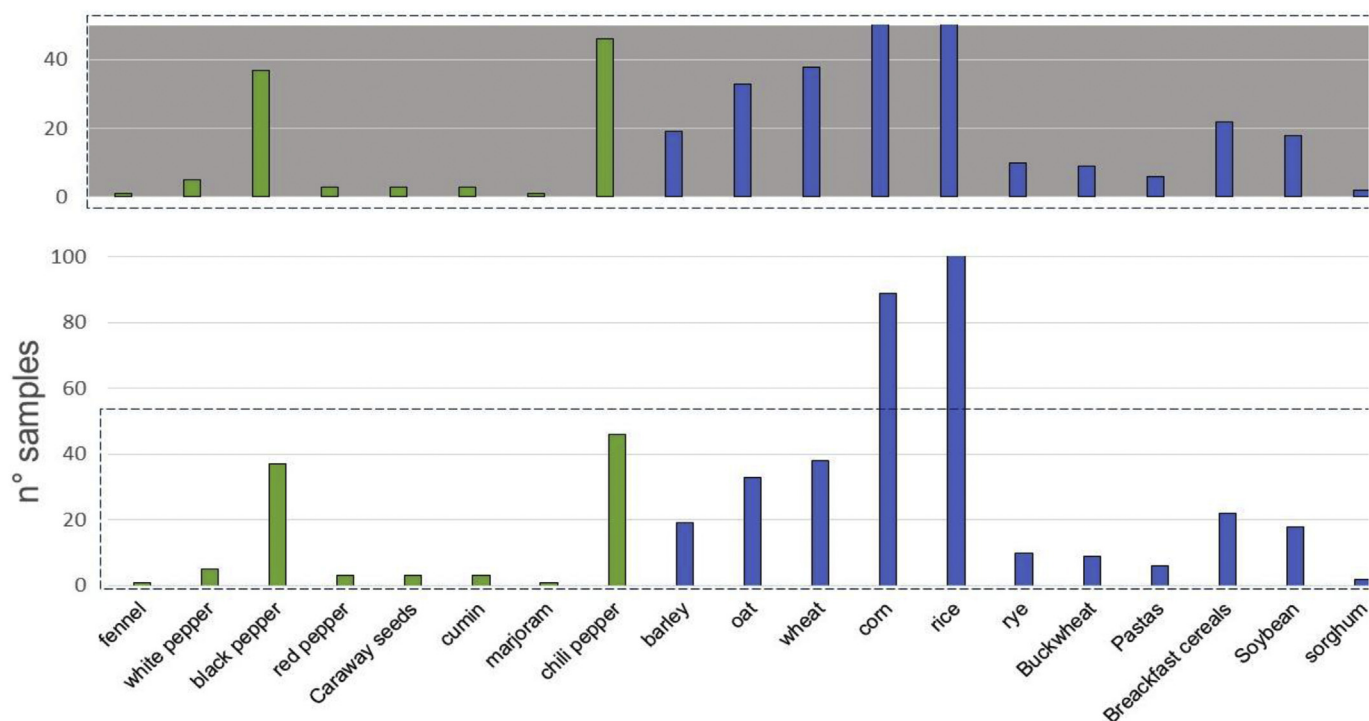
Fig. 5. Samples of different nature and quantities where STE was found. Spices (green), cereals (blue). The shaded area corresponds to the enlarged region. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

with AFB_1 and aflatoxin B_2 (AFB_2). STE was detected in samples of chili that were collected in Sri Lanka and Belgium. STE appears in these samples in a concentration range from 11 to $32 \,\mu g \, kg^{-1}$. AFB_1, ochratoxin A (OTA), fumonisin B_2, citrinin and alternariol monomethyl ether mycotoxins were also found. The co-occurrence of STE-AFB_1 was in three samples and STE-AFB_1-OTA was in two samples (Yogendrarajah et al., 2014b). STE was also found in black and white pepper in Sri Lanka (Yogendrarajahet al. 2014a). Only 20% of the samples with STE were contaminated with some aflatoxins. A fungal study of the samples revealed that *A. flavus*, *A. parasiticus, A. niger* and *Penicillium* spp were the dominant fungi. The authors concluded that pepper can disrupt the aflatoxin biosynthesis.

With regard to cereals, STE was detected in Norway in barley, oat and wheat, with average concentrations of 1, 2.1 and $0.1 \,\mu g \, kg^{-1}$ respectively (Uhlig et al., 2013). STE was found in barley, wheat, buckwheat, rye in Latvia (at a concentration range from 0.7 to $83 \,\mu g \, kg^{-1}$) (Veršilovskis et al., 2008a). Wheat, corn and rice contaminated with STE were also found in China (Tian and Liu, 2004). Results showed that average concentrations of STE in wheat, corn and rice were $68.9 \,\mu g \, kg^{-1}$, $32.2 \,\mu g \, kg^{-1}$, and $13.9 \,\mu g \, kg^{-1}$, respectively. In Syria STE was found in wheat samples, at an average concentration of $1.4 \, ng \, mL^{-1}$ (Alkadri et al., 2014).

A study carried out in European countries (Greece, Italy, The Netherlands, United Kingdom, Cyprus, Germany, Latvia, Lithuania and Poland) revealed the presence of STE in grains (soft and hard wheat, rye, corn, rice, barley, oat), in cereal products that have to be cooked beforehand (ground wheat, ground corn, ground barley, rice and pasta) and cereal products that they are consumed without treatment (breakfast cereals and cereal-based on infant food) (Mo et al., 2015).

STE was detected in white rice and sorghum samples. The samples were sourced from Korean markets (Ok et al., 2016). In Italy, the occurrence of STE was detected in paddy rice samples, in the $0.29–15.85 \,\mu g \, kg^{-1}$ concentration range. In processed rice, a widespread contamination was found in brown and parboiled rice. All the brown rice samples were contaminated between 0.12 and $1.32 \,\mu g \, kg^{-1}$; for parboiled rice, the incidence was 90.9% and the maximum level was

$1.09 \,\mu g \, kg^{-1}$ (Bertuzzi et al., 2017).

STE was detected in corn, ground nuts and feed from Burkina Faso and Mozambique, at an average concentration of 2.3, 0, $6.5 \,\mu g \, kg^{-1}$ and 2.7, 9.7, $11 \,\mu g \, kg^{-1}$, respectively (Warth et al., 2012).

In Ivory Coast, STE was detected in rice and peanut paste, with average concentrations of 4.7 and $8.1 \,\mu g \, kg^{-1}$ (Manizan et al., 2018). STE was found in corn, ground nuts, ground nuts soap, kuru-kuru and dagwa from Cameroon, with average concentration of 2.0, 5.0, 0.6, 1.0 and $1.4 \,\mu g \, kg^{-1}$ respectively (Abia et al., 2013). In Nigeria (Ogara et al., 2017) and Japan (Nomura et al., 2018), STE was found in corn, with an average concentration of 121 and $1.0 \,\mu g \, kg^{-1}$, respectively. In Japan, STE was also detected in soybean meal and formula feed at a concentration of 0.63 and $0.97 \,\mu g \, kg^{-1}$, respectively (Nomura et al., 2018).

STE was found in 2 beer samples in Latvia (Veršilovskis et al., 2008b), in rye, rye-wheat and wheat bread (Veršilovskis and Bartkevičs, 2012) with a concentration range of $2–7 \,\mu g \, kg^{-1}$. Furthermore, the authors determined that STE is stable in the process of baking bread, when it is prepared from flour that comes from wheat grains contaminated with STE.

STE was detected in traditional Chinese medicine, which is largely based on natural plants. These medicinal products were obtained from pharmacies and hospitals (Zheng et al., 2014).

STE exposition via inhalation should be considered in view of the induction of pulmonary adenomas (see Section 2.2). Fungal spores are regularly found in outdoor and indoor air. Indoor exposure to STE may occur by ingestion of house dust and by inhalation of fungal spores (deposition in the lower respiratory system and interactions with lung tissue) (Engelhart et al., 2002; Despot and Klarić, 2014). *A. versicolor* is a common indoor fungus in damp houses (Nielsen et al., 1999; Tuomi et al., 2000; Engelhart et al., 2002; Despot and Klarić, 2014). *A. versicolor* was implicated as causes of building-associated pulmonary disease in a courthouse in Florida. The quantification of STE was not possible because of interfering peaks on HPLC-UV (Hodgson et al., 1998). In a study of interior finishes of Finnish buildings with moisture problems contaminated with fungi, it was found that the most present toxin was STE ($0.2–310 \,\mu g \, kg^{-1}$) (Tuomi et al., 2000). Nielsen et al. (1999) found
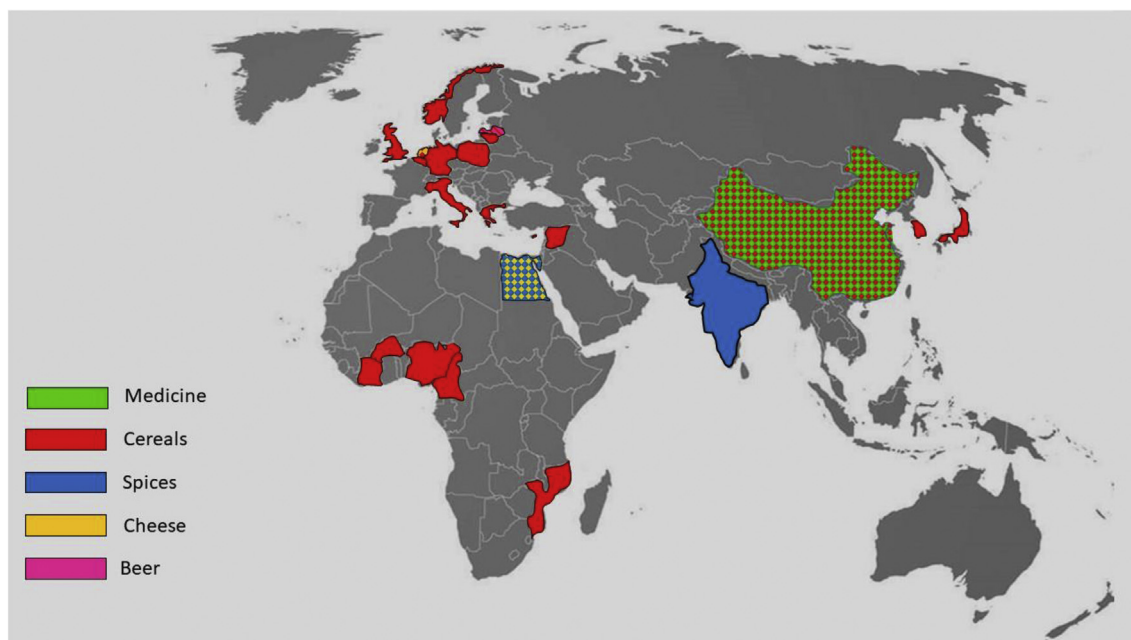
Fig. 6. Countries where STE was found.

STE in infested wallpaper (0.002–2 µg cm^{-2}). Red colored areas infested with none or poorly sporulating *A. versicolor* biomass contained the largest quantities of STE, whereas areas with many *A. versicolor* conidia contained very small quantities. Engelhart et al. (2002) did a study to determinate *A. versicolor* isolated and STE in native carpet dust from damp dwellings with mold problems. Carpet dust samples contained *A. versicolor* (median, 3.1×10^4 CFU/g) and STE was detected between 2.0 and 3.8 µg kg^{-1}.

Fig. 6 shows the different countries where STE was found. Each country is marked with a different color according to the food where STE was found.

2.4. Prevention and control

The prevention and control of STE focuses on limiting the growth of fungi during the food processing process, from the field to the time of consumption.

The Ministerio de Agricultura, Alimentación y Medio Ambiente of Spain published in 2014 a Good Practices guide to prevent aflatoxin contamination in primary production (MAgrAMA, 2014). Thus, a list of measures to be adopted in both the field and in storage and transport phases and in the livestock farms is present.

Buchanan et al. (1983) found that caffeine inhibited the growth of *A. versicolor* and thus decreased the STE production. No toxin was detected in cultures that contained more than 2 mg of caffeine per mL.

Abdel-Wahhab et al. (2005) investigated the efficacy of Egyptian montmorillonite (EM), a clay mineral, to adsorb STE. Nile tilapia fish was used as an in vivo model to evaluate the protective effect of EM against STE-induced toxicity and clastogenicity. The results showed that EM had a high capacity of adsorbing STE, ranged from 93.1 to 97.8% of the available STE in aqueous solutions. The authors concluded that EM itself was safe and successful in the prevention of STE toxicity and clastogenicity.

Kocić-Tanackov et al. (2012) investigated the effect of oregano extract on the biosynthesis of STE by *A. versicolor*. Significant reductions in STE biosynthesis during 21 days of incubation were observed for extract concentrations higher than 0.06 mL of extract/100 mL solution. These results showed that the oregano extract could be used as a food preservative to prevent food-borne fungal infections and STE production.

2.5. Analytical methods for STE quantitative determination

Three groups of analytical methods to determine STE in food can be mentioned: chromatographic methods, ELISA immunoassays and chemical sensors. Independently from the methods employed, all analytical methods used to determine mycotoxins require a combination of procedures such as sampling, sample preparation, clean-up and quantitative analysis.

2.5.1. Sample extraction

For grain extraction, finely ground sample was extracted with ACN/water (84:16, v/v). The sample was homogenized using a shaker during 30 min and then centrifuged or filtered. Then, n-hexane was added to ACN/water mixture, for the analysis of STE in cheese samples. This solvent is added to remove the cheese fat. The sample was homogenized using a shaker during 30 min and then centrifuged. ACN/water phase was the phase used. A new extraction method has been developed. It is based on QuEChERS, which stands for Quick, Easy, Cheap, Effective, Rugged and Safe (Arroyo-Manzanares et al., 2015; Liu et al., 2016; Cao et al., 2018; Manizan et al., 2018; Reichert et al., 2018). It involves a previous extraction based on partitioning via salting-out between aqueous and organic layer, and a subsequent clean up based on dispersive SPE (dSPE) by combining MgSO$_4$ and different sorbents (i.e. C18, primary and secondary amine, etc.) (Arroyo-Manzanares et al., 2015).

2.5.2. Clean-up

The most used methods for sample clean-up, are solid phase extraction (SPE), consisting of non-specific sorbent (reverse-phase, normal-phase, ion exchange, etc.), and immunoaffinity columns (IAC) that use very selective binding materials. Table 4 shows the different clean-up columns used with their respective methods of conditioning, load, washing and elution.

2.5.3. Chromatographic methods

The most commonly used methods to determine STE in food are chromatographic methods. Versilovskis and De Saeger (2010) reported a review, which describes the chromatographic methods to determine STE in food until the year 2010. These methods include from TLC to HPLC with UV-visible or MS/MS detector.

Table 4
Different clean-up columns used to determine STE.

column	Samples	Condition	Load	Washing	Elution	Reference
Strata X, SPE	Wheat, buckwheat, barley, oats, rye, cheese and beer	- 3–6 mL methanol - 3–6 mL H_2O	Extract diluted with H_2O	- ACN: H_2O (40:60, v/v) - Methanol: H_2O (40:60, v/v)	- 3 mL ACN 100%	Veršilovskis et al., 2007; Veršilovskis et al., 2008a; Veršilovskis et al., 2008b; Veršilovskis et al., 2009
Easy-Extract sterigmatocystin®, IAC	Maize, wheat, oats, rye, rice, sunflower seeds, cheese and beer		Extract diluted with PBS solution	- 10 mL PBS - 10 mL H_2O	- 2 mL ACN 100%	Bertuzzi et al., 2017; Marley et al., 2015
AFLAKING, IAC	Wheat, corn, barley, rice and buckwheat		Extract diluted with PBS solution	- 10 mL PBS - 10 mL H_2O	- 3 mL ACN 100%	Hossain and Goto, 2015
QuEChERS + HLB SPE	Rice, wheat and barley, infant cereals and Beer	- 6 mL methanol - 6 mL H_2O	Extract diluted with H_2O	- 6 mL methanol: H_2O (70:30 v/v)	- 6 mL ACN 100%	Liu et al., 2016
Oasis HLB™, SPE	Food supplements	- 10 mL CH_2Cl_2/methanol (80:20, v/v) + 50 mM formic acid. - 5 mL methanol - 20 mL 10 mM HCl - 10 mL H_2O	Extract diluted with H_2O	- 10 mL H_2O	- 1 mL methanol + 4 mL CH_2Cl_2/methanol (80:20, v/v) containing 50 mM formic acid.	Di Mavungu et al., 2009

Chromatography methods were developed to determine STE in different cereals: Therefore, the GC-MS (with a limit of detection (LOD) of 6 pg, which is equivalent to 2.4 μg kg^{-1} in grains) had been published (Hossain and Goto, 2015). HPLC methods with UV-DAD detector (LOD of 0.74 μg kg^{-1}) (Marley et al., 2015; Liu et al., 2016) and with MS/MS detector (LOD between 0.05 and 0.2 μg kg^{-1}) (Marley et al., 2015; Bertuzzi et al., 2017), UHPLC-ESI-MS/MS (LOD of 0.11 μg kg^{-1}) (Liu et al., 2016) and LC-MS (LOD of 2.5 pg, which is equivalent to 1.0 μg kg^{-1} in the product) (Sasaki et al., 2014) have been published.

Chromatography methods for the simultaneous determination of STE and AFB$_1$ were developed for different samples such as beer, LC-MS/MS (LOD of 0.03 μg kg^{-1}) (Zhao et al., 2017a) and cereals, LC-ESI-MS/MS (LOD of 0.3 μg kg^{-1}) (Nomura et al., 2018), LC-MS/MS method (LOD of 0.02 μg kg^{-1}) (Zhao et al., 2017b). An inter-laboratory study was performed in eight laboratories to validate a LC/MS/MS method (Ok et al., 2016).

Multi-mycotoxin chromatographic methods, where STE has been included, were developed to detect mycotoxins in different food. Therefore, UHPLC-MS/MS was applied for the determination of 17 mycotoxins (the LOD for STE was 1 μg kg^{-1}) (Li et al., 2018), 77 mycotoxins (the LOD for STE was 0.62 μg kg^{-1}) (Manizan et al., 2018) and 10 mycotoxins (the LOD for STE was 0.14 μg kg^{-1}) (Arroyo-Manzanares et al., 2015). The LC-MS/MS was applied for the determination of 15 mycotoxins (LOD for STE was 0.125 ng mL^{-1}) (Flores-Flores and González-Peñas, 2017), 36 mycotoxins and 93 microbial secondary metabolites (LOD for STE was 0.1 μg kg^{-1}) (Ogara et al., 2017) and 11 mycotoxins (LOD for STE was between 0.03 and 0.05 μg L^{-1}) (Cao et al., 2018). The LC-ESI-MS/MS was applied for the determination of 320 toxic and potentially toxic fungal secondary metabolites (LOD for STE was 0.15 μg kg^{-1}) (Abia et al., 2013), 131 pesticides and 35 mycotoxins (LOQ for STE was 100 μg kg^{-1}) (Reichert et al., 2018) and 247 fungal and bacterial metabolites (LOD for STE was 2 μg kg^{-1}) (Warth et al., 2012).

2.5.4. Immunoassays

Immunoassays are bioanalytical methods in which the quantization of the analyte depends on the reaction between an antigen (analyte) and an antibody. Mainly, these methods are based on a competitive binding reaction between a fixed amount of labeled form of an analyte and a variable amount of unlabeled sample analyte for a limited amount of binding sites on a highly specific anti-analyte antibody (Darwish, 2006).

An indirect competitive enzyme-linked immunosorbent assay (ic-ELISA) method and lateral-flow immunochromatographic assay (ICA) strip method were developed for the detection of STE in cereal products (Kong et al., 2017). This is a rapid, simple and instrument-free diagnostic tool for on-site screening with qualitative/semi-quantitative detection, and the results can be obtained within 5–10 min.

Sensitive and specific anti-sterigmatocystin monoclonal antibody (mAb) 4G10 was obtained by immunization and cell fusion (Wang et al., 2009). The 50% inhibition concentration and limit of detection for the ic-ELISA method were 0.092 and 0.015 ng mL^{-1}, respectively. The visual limit of detection (vLOD) and cut-off value for the lateral-flow ICA strip method were 0.1 and 0.5 ng mL^{-1}, respectively. From the analysis of different cereal samples (wheat, maize and rice), the recovery rates ranged from 78.3% to 122.0%.

Wegner et al. (2017) developed a competitive direct enzyme immunoassay (EIA) for STE detection. Besides, a simplified method to produce specific polyclonal rabbit antibodies against STE was established, using a STE-glycolic acid-ether derivative (STE-GE) conjugated to keyhole limpet haemocyanin (immunogen). The standard curve measuring range for STE-EIA was 0.17–2 ng mL^{-1}, with a LOD (20% binding inhibition) of 130 pg mL^{-1}. The test was highly specific for STE.

Oplatowska-Stachowiak et al. (2018) developed a direct competitive enzyme-linked immunosorbent assay (dc-ELISA) for the STE detection in rice, wheat and corn. Cut offs for rice, wheat and corn were 1.2, 1.2 and 1.3 μg kg^{-1}. The cross-reactiviy with aflatoxins B$_1$, B$_2$, G$_1$, G$_2$ and M$_1$ was below 1%.

2.5.5. Chemical sensors and biosensors

A chemical sensor is defined as a device which responds to a particular analyte in a selective way through a chemical reaction and can be used for the qualitative or quantitative determination of the analyte (Eggins, 2002).

When operated, a chemical sensor performs two functions, recognition and transduction. First, the analyte interacts in a more or less selective way with the recognition (or sensing) element, which shows affinity for the analyte. As a result of the analyte interaction with the sensing element, certain physical or chemical properties of the sensing element vary as a function of the analyte concentration. In order to allow the user to assess this variation, the transductor converts the above change into a measurable physical quantity (Bănică and Advisor, 2012).

Biosensors are really a sub-set of chemical sensors, but they are often treated as a topic in their own right. A biosensor can be defined as a device incorporating a biological sensing element connected to a transducer (Eggins, 2002; Bănică and Advisor, 2012).

In the literature, two chemical sensors and three biosensors can be found for STE detection. A sensitive fluorescent sensor for determination of STE based on carbon dots-embedded molecularly imprinted polymer (CDs@MIP) was developed (Xu et al., 2016). The CDs acted as antennas for signal amplification and optical readout, and the MIP coated on the CDs surface provided specific binding sites for STE. Under optimized conditions, the relative fluorescence intensity of CDs@MIP decreased linearly with the concentration of STE from 0.05 to 2.0 mg L^{-1}, with a LOD of 0.019 mg L^{-1} (S/N = 3). The sensor was also used to determine the content of STE in corn, rice and millet samples. Recoveries in sample were in the range of 92–102%.

Molecularly imprinted polymers (MIPs) were fabricated to recognize trace STE with high selectivity and sensitivity (Liu et al., 2017). These authors used the hexagonal phase up conversion fluorescent nanoparticles (β-NaYF4: Yb^{3+}, Er^{3+}) and SiO$_2$ as fluorescent carrier, UCNP@SiO$_2$. Under optimal conditions, the fluorescence enhancement of MIPs increased as the concentration of STE increased. Fluorescence enhancement versus STE concentration showed a good linear relationship in the range of 0.02–1.0 mg L^{-1}, with a detection limit of 0.013 mg L^{-1}. Recoveries in sample were in the range of 83.8–88.8% for rice, 82.1–87.5% for maize, and 80.6–89.2% for soybeans.

Yao et al. (2006) reported a novel biosensor; whose working electrode is constructed by enzyme (aflatoxin–detoxifizyem (ADTZ)) modified multi-walled carbon nanotubes (MWNTs). The differential pulse voltammetry (DPV) technique was used to obtain the relationship between the response current and the STE concentration. The linear detection range for STE was from 0.13 to 4.29 × 10^{-6} mol L^{-1}, with a LOD of 0.13 × 10^{-6} mol L^{-1}.

Chen et al. (2010) developed a simple, rapid and highly sensitive electrochemical biosensor for the STE detection. The biosensor is based on a new enzyme named aflatoxin-oxidase (AFO), that was immobilized onto chitosan-single-walled carbon nanotubes (CS-SWCNTs) modified Au electrode via electrostatic and hydrophobic interactions. The enzymatic electrode exhibited an excellent electro-catalytic response to STE, long-term stability, and good reproducibility. The linear range for STE determination was from 10 to 1480 ng mL^{-1}, with a LOD of 3 ng mL^{-1} based on the signal-to-noise ratio of 3. The kinetic parameters such as α (charge transfer coefficient), k$_s$ (electron transfer rate constant) and K$_{M,app}$ (the apparent Michaelis–Menten constant) were 0.4, 0.9 ± 0.02 s^{-1} and 7.8 × 10^{-6} mol L^{-1}, respectively. Chronoamperometry was the technique used, at a potential of - 0.4 V, with a response time of 10 s.

A third-generation enzymatic electrochemical biosensor for the determination of STE has very recently developed by us (Díaz Nieto, 2017; Fernández et al., 2017). The biosensor is based on the use of a composite obtained from soybean peroxidase enzyme (SPE) and chemically-reduced graphene oxide (CRGO) deposited on GC electrodes. Amperometric measurements were performed at a potential of - 0.090 V vs. Ag/AgCl. The biosensor shows a linear response in the concentration range from 6.9 × 10^{-9} mol L^{-1} to 5.0 × 10^{-7} mol L^{-1}. The LOD was 2.3 × 10^{-9} mol L^{-1}, and the apparent Michaellis-Menten constant, K$_M^{app}$, was 1.5 × 10^{-6} mol L^{-1}. The biosensor was also used to determine STE in corn samples contaminated with the A. flavus fungus.

3. Conclusions

Sterigmatocystin can be produced by fungal species phylogenetically and phenotypically different. Its biosynthetic path is the best known and most studied. This mycotoxin is synthesized through the acetate-malonate pathway. Sterigmatocystin is a carcinogenic compound that has been demonstrated to affect several species of experimental animals.

Sterigmatocystin can be found in cheeses, cereals, spices, and beer, bread, housing and building materials and in traditional Chinese medicine.

The prevention and control of STE focuses on limiting the growth of fungi during the food processing process, from the field to the time of consumption.

Analytical methods for the determination of STE in foodstuffs are based on chromatographic, immunoassays, chemical sensors and biosensors.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.fct.2018.05.057.

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