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REVIEW

Sterigmatocystin: Occurrence in foodstuffs and analytical methods – An overview

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Sterigmatocystin (STC) is a mycotoxin produced by fungi of many different *Aspergillus* species. Other species such as *Bipolaris, Chaetomium, Emiricella* are also able to produce STC. STC producing fungi were frequently isolated from different foodstuffs, while STC was regularly detected in grains, corn, bread, cheese, spices, coffee beans, soybeans, pistachio nuts, animal feed and silage. STC shows different toxicological, mutagenic and carcinogenic effects in animals and has been recognized as a 2B carcinogen (possible human carcinogen) by International Agency for Research on Cancer. There are more than 775 publications available in Scopus (and more than 505 in PubMed) mentioning STC, but there is no summary information available about STC occurrence and analysis in food. This review presents an overview of the worldwide information on the occurrence of STC in different foodstuffs during the last 40 years, and describes the progress made in analytical methodology for the determination of STC in food.

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1 Introduction

Sterigmatocystin (STC) is a fungal secondary metabolite produced by many *Aspergillus* species such as *A. versicolor*, *A. chevalieri*, *A. ruber*, *A. amstelodami*, *A. aureolatus*, *A. quadrilineatus* and *A. sydowi* [1–5]. Other species such as *Penicillium*, *Bipolaris*, *Chaetomium*, *Emiricella* are also able to produce this mycotoxin [6–14]. The main STC producer, *i.e. A. versicolor,* is generally xerophilic, which means it can grow at low water activity (<0.8). The minimum and maximum growth temperatures for *A. versicolor* are 4 and 40°C with an optimum at 30°C. Its optimal water activity is 0.95 with a minimum at 0.75. Optimal conditions for STC production (by *A. versicolor* and *Bipolaris sorokiniana*) are temperatures between 23 and 29°C, water activity starting from 0.76 and a moisture content above 15% [15–17]. STC is a biogenic precursor of aflatoxin B_1 (AFB_1) (Fig. 1) [18–20]. Although STC has frequently been detected in different foodstuffs, there is no summarized information about its occurrence and analysis in food.

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Abbreviations: AFB_1, aflatoxin B_1; **LD_{50},** dose of a toxic substance (mg/kg body weight) which kills 50% of the test animals; **MCF,** maximal concentration found; **STC,** sterigmatocystin

1.1 Chemical and physical properties

STC crystallizes as pale yellow needles and is readily soluble in methanol, ethanol, ACN, benzene and chloroform. It reacts with hot ethanolic potassium hydroxide and is methylated by methyl sulphate and methyl iodide. Methanol or ethanol in acid produces dihydroethoxysterigmatocystin. The melting point of STC is 246°C; maximum absorptions in ultraviolet light are at 245 and 325 nm; summary molecular formula is $C_{18}H_{12}O_6$ (elemental analysis: C–66.67%; H–3.73%; O–29.60%); molecular mass is 324.284 g/mol; CAS number is 10048-13-2; STC proton nuclear magnetic resonance (^{1}HNMR) data obtained in deuterochloroform (270 MHz): $\delta = 4.00$ (s,3 H); $\delta = 4.82$ (d of t, 1 H, J (coupling constant in hertz) = 7.0, 2.0 Hz); $\delta = 5.76$ (t, 1 H, $J = 2.4$ Hz); $\delta = 6.45$ (s, 1 H); $\delta = 6.51$ (t, 1 H, $J = 2.4$ Hz) $\delta = 6.77$ (d, 1 H,

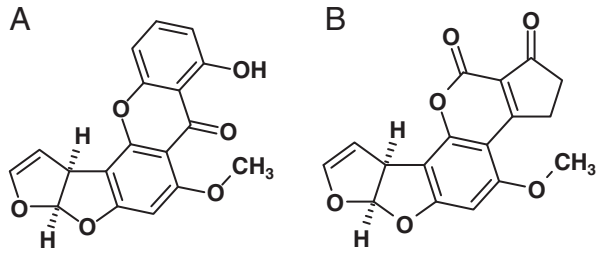

Figure 1. Chemical structures of STC (A) and AFB_1 (B).

$J = 8.4$ Hz); $\delta = 6.840$ (d, 1 H, $J = 7.0$ Hz); $\delta = 6.843$ (d, 1 H, $J = 8.4$ Hz); $\delta = 7.52$ (d, 1 H, $J = 8.4$ Hz); $\delta = 13.15$ (broad singlet, 1 H) [21]. STC has a structural relationship to AFB_1; it contains the same 7,8-dihydrofuro[2,3-*b*]furan moiety, which for STC is fused to a hydroxyl- and methoxy-substituted xantone [21] (Fig. 1). There is only one report available on the stability of STC in solvents [22], where it is similar to the aflatoxins, and few reports are available on the stability of STC in cheese under different storage temperatures and time periods [23, 24], where it is found to be stable in various temperatures for 6 months.

1.2 Toxicity, carcinogenity, mutagenity and teratogenity

Acute toxicity, mutagenity, cytotoxicity and carcinogenity of STC have been thoroughly researched [9, 25, 26]. STC is generally recognized as a potential carcinogen, mutagen and teratogen [27, 28]. STC is acutely toxic to the liver of most animals tested [29, 30] and its carcinogenicity has been demonstrated with organ specificity varying with species and route and frequency of administration [25, 30–33]. In rats STC induces hepatocellular carcinomas after oral administration [31] or intraperitoneal injection [33] and squamous cell carcinomas after repeated application to the skin [30]. Despite its potent toxic and carcinogenic properties in animals, the importance of STC as a human health hazard is unknown. Surveillance programs have regularly detected its presence in foods, but mostly at low concentrations (ppb range) even though STC-producing fungi are widely distributed [34]. Nonetheless, STC is of interest as a model compound for cancer induction because of its structural similarity to AFB_1. In rats and monkeys, the lethal potency of STC is about 1/10th that of AFB_1 [29, 35], and STC is between 1 and 2 orders of magnitude less potent as a hepatocarcinogen for the rat [25, 31]. A comparable quantitative difference exists in the toxicity and mutagenity of STC and AFB_1 in *Salmonella typhimurium* [36–38]. Toxic effects induced by STC are mostly similar to effects induced by AFB_1 [39]. STC is toxic to kidneys [40] and also hepatotoxic and hepatocarcinogenic, causing liver cancer in animals [41–45].

STC is associated with acute clinical symptoms of bloody diarrhea and death in dairy cattle fed with feed containing *A. versicolor* and high levels of STC of about 8 mg/kg [46]. It is less acutely toxic than AFB_1 to rodents and monkeys, but appears to be slightly more toxic to zebra fish [47] (http://services.leatherheadfood.com/mycotoxins/item.-asp?number = 12&fsid = 17&mytype = Basic). The dose of a toxic substance (mg/kg body weight) which kills 50% of the test animals (LD_{50}) in mice is in excess of 800 mg/ kg. The 10-day LD_{50} in Wistar rats is 166 mg/kg in males and 120 mg/kg for administration in females. The 10-day LD_{50} for vervet monkeys is 32 mg/kg. Chronic symptoms include induction of hepatomas in rats, pulmonary tumours in mice, renal lesions and alterations in the liver and kidneys of African Green monkeys. Rats fed 5–10 mg/kg of STC for 2 years showed a 90% incidence of liver tumours [47]. STC causes tumours on experimental animal skin after 70 days of dermal application [30]. The STC mutagenic activity is several times higher than induced by aromatic hydrocarbones [48]. Mammalian cells in culture exposed to STC display nucleolar aberrations, inhibited mitosis, inhibited uptake of thymidine and uridine, and stimulated DNA repair synthesis [49–52]. STC also has been demonstrated to inhibit RNA synthesis in rat liver [53].

As listed above there are several structural similarities between AFB_1 and STC, but in contrast to the large literature on AFB_1, little information has been published on metabolism and biochemical effects of STC. Dimensions and absolute configuration of the bisdihydrofuran moiety are very similar in both molecules [39]. This suggests a metabolic activation on the same site, the C_2–C_3 double bound, as in AFB_1 [39, 54]. It has been shown that metabolic activation (epoxide formation by liver microsomes P450s) is required for the toxicity and mutagenicity of STC in bacteria, animals and some cultured cells [35–38, 48, 55]. The mechanism of STC mutagenicity is linked to its covalent binding to the DNA molecule after metabolic activation and its conversion to STC–N^7–Guanine adduct after hydrolysis (Fig. 2) [54]. The structure of the STC adduct at the *N*-guanine of oligodeoxynucleotides was determined [56].

The acute toxicity, carcinogenicity and metabolism of STC were compared with those for aflatoxin and several other hepatotoxic mycotoxins and it was classified as a 2B carcinogen by the International Agency for Research on Cancer [57–59].

1.3 Legislation and control in foodstuffs

Czech Republic and Slovakia have set regulations on STC at a level of 5 μg/kg for rice, vegetables, potatoes, flour, poultry, meat, milk, and 20 μg/kg for other foods [60]. Other countries have no legislation for STC and therefore no official control/monitoring programmes. However, soon after STC was recognized as a highly toxic compound, the California Department of Health Services used (threshold dose 50 or carcinogenic potency) chronic dose rate (expressed in mg/kg of bodyweight *per* day) which would induce tumours in half

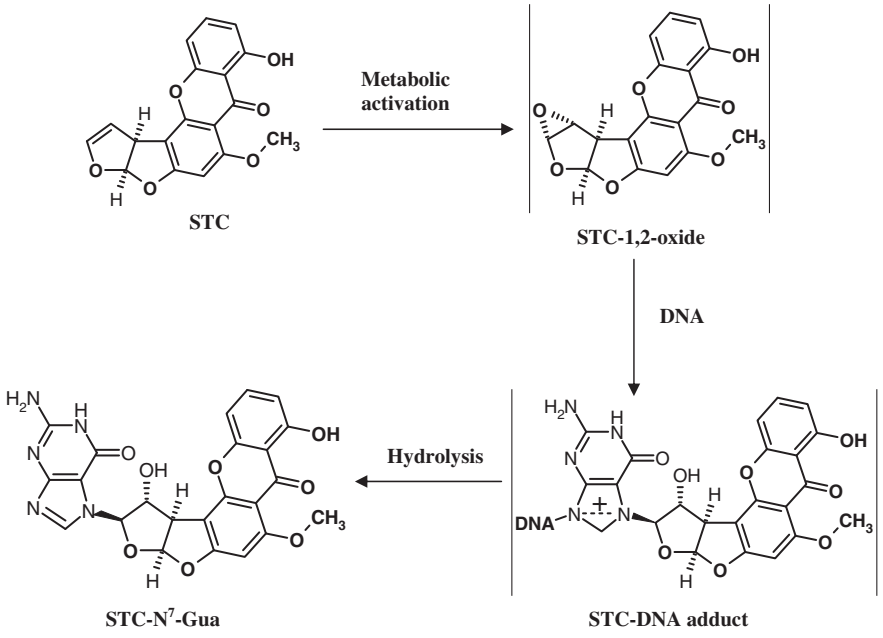

Figure 2. Scheme of metabolic activation and DNA binding of STC by Essigmann [54].

(50%) of the number of test animals at the end of the standard lifespan for the species values from the Cancer Potency Database to produce 'no significant risk' intake levels for humans. The level resulting was 8 μg/kg of body weight *per* day for a 70 kg adult [47].

2 STC occurrence in foodstuffs

2.1 Occurrence data

The natural occurrence of STC in food and raw commodities was reported. Also, relatively high levels (ppm range) of STC were detected in dwellings and building materials (also in indoor air) contaminated by *A. versicolor* [61–65]. Contamination of cereals with *Aspergillus* fungi under European (north, north-west, northeast and middle Europe) climatic conditions is possible mainly during the storage period. Post-harvest contamination of cereals with *Aspergillus* fungi involves a health risk due to the possible production of STC and other mycotoxins such as AFB_1 and ochratoxin A [66].

STC was reported as a fungal metabolite in mouldy wheat, rice, barley, rapeseed, peanut and corn [17, 67–71]. After inoculation with *A. versicolor*, high levels of STC were produced in bread, cured ham and salami (up to 160–180 μg/kg in the 7th drying day if salami was dried at 20–28°C) [72]. During investigations with different types of bread (wheat, rye, whole grain wheat *etc.*) inoculated with *A. versicolor* spores, it was found that the concentration of STC could reach 100–400 μg/kg on the 10th storing day [73].

During the last 30–40 years only a few surveys on the natural occurrence of STC in different foods were carried out. During the period 1976–1979, just over 4000 samples of animal feedstuffs comprising cereals, compound feeds, hay and silage were examined for moulds and mycotoxins. This monitoring was carried out in the course of routine advisory and investigational work undertaken by the Agricultural Development and Advisory Service (Ministry of Agriculture, Fisheries and Food) Microbiology Laboratories in England and Wales in connection with livestock health and production problems and defects in grain storage. Moldy cereals, mostly invaded by *Penicillium* and *Aspergillus* species, were often found contaminated with ochratoxin A, citrinin, zearalenone and STC (17 out of 523 grain samples and one hay sample were found positive for STC) [74]. The drawback of this survey was the high LOD (20 μg/kg) of the analytical method. Another survey was done in Brazil [75], in the same decade, where STC was not detected in 286 analyzed samples (corn, cassava flour, rice and dried beans). Again the LOD of the applied method was rather high (15–35 μg/kg). In 1986 about 167 corn samples were analyzed in Turkey [76]. STC was detected in 10 samples at levels close to the LOD of 20 μg/kg. Further, STC was found in wheat in Canada in 1972 (maximal concentration found (MCF) – 300 μg/kg) [67]; in rice from Japan in 1975 (MCF – 16 300 μg/kg) [77, 78], in animal feed (MCF – 100 μg/kg) [79] and in coffee beans (MCF – 12 000 μg/kg) [80] in Poland in 1977; in corn in India in 1982 (MCF – 100 μg/kg) [81]; in ten samples of red pepper, caraway, cumin and marjoram (concentration range 18–23 μg/kg) in Egypt in 1994 [82]. Another study was carried out in the UK in 1996 in which a new LC-MS method was used for STC determination (LODs between 1.7 and 2.4 μg/kg) in cheese, bread and corn products. No positive samples were found [83]. In 2006–2007 STC was found in 55 out of 215 samples (concentration range 0.7–83 μg/kg) of different grains

(barley, wheat, buckwheat, rye) in Latvia [84]. There is only one report on STC in beer where it was detected in 2 out of 26 analyzed beer samples (concentration range 4–7.8 μg/kg) [85]. Less significant reports are also available on the occurrence of STC in small quantities or isolated from fungi found in foodstuffs: grains and bread [67, 68, 86, 87], soybeans and groundnuts [86, 88], rice [86], cocoa beans [89], vegetables [90], pistachio nuts [91], coffee beans [92, 93] and feed [46, 94]. To enable risk analysis more STC occurrence data are needed in the EU and other countries in the world.

2.2 Occurrence in cheese

The presence of mycotoxins, including STC, in dairy products can have two origins: (i) indirect contamination, which results from lactating animals ingesting contaminated feed and (ii) direct contamination, which occurs because of intentional or accidental growth of moulds on dairy products [23]. *A. versicolor* was frequently present on cheese, while other aflatoxin producers such as *A. flavus* and *A. parasiticus* were seldom found. *A. versicolor* isolates from cheese and air in the cheese ripening room were all able to produce STC [3]. As cheese can easily become moldy during the ripening process in warehouses, and during storage after cutting and slicing in shops or at home, there is a high possibility that it becomes contaminated with STC.

STC was detected in cheese contaminated with *A. versicolor* [95]. Northolt *et al.* reported the occurrence of STC in cheese (Gouda and Edam) ripening in warehouses in the Netherlands. The STC contaminated cheeses originated from eight warehouses and were found in all categories of cheese age (2–8 months) in a concentration range of 5–600 μg/kg [96]. STC was stable in contaminated cheeses for a 3 months period at various temperatures (−18 to +16°C) [23, 97]. However, low temperatures (5–7°C) should prevent growth of *A. versicolor* and production of STC during ripening and storage. At these temperatures, the moulds that develop will be *Penicillium* species, which do not produce aflatoxins or STC [98]. However, cheese-ripening temperatures depend on individual cheese making technologies.

Occurrence, distribution and stability of STC were also studied in Ras cheese in Egypt. Thirty-five percent was found positive with a mean concentration of 22 μg /kg (range 10–63 μg/kg). In Ras cheese contaminated with spores of *A. versicolor*, toxin production started after 45 days of ripening and reached a maximum after 90 days. Aged cheese (more than 6 months) inhibited toxin production [24].

The migration studies of STC in Wilstermarsh cheese after inoculation with *A. nidulans* and incubation at 15°C or room temperature for 40 days was limited to 0.5 cm from the cheese surface [99]. Similar results were obtained with six naturally contaminated Gouda cheeses on which *A. versicolor* was growing [23]. Veringa *et al.* found that STC was in 1–1.5 cm from the cheese surface [100]. In another migration study, no STC was detected in the centre part of three naturally contaminated hard cheeses that contained 45–330 μg/kg in the outer 2 cm layer [101].

In 1989 studies were carried out in the Netherlands on factors that affect STC development in cheese. Lactose, glycerol, fat content, high humidity during ripening (>86%) and temperature were defined as a first group of stimulating factors in STC production by *A. versicolor* on cheese [100]. The toxin was produced on cheese plasticoated once during the first 5–6 wk of ripening at 14°C and 90% relative humidity. STC concentration after 8 days of ripening could reach 160–700 μg/kg depending on the stimulating factors. On cheeses plasticoated twice the production of STC was clearly reduced. Apparently a thick plastic coating on the cheese surface allows diffusion of low-molecular substances necessary for growth of the mould, but probably inhibits diffusion of high-molecular substances, fat and fat-like compounds, which are important in the production of STC. Therefore, the presence of several layers of plastic coating on the cheese surface during the first weeks of ripening is probably an effective remedy against production of the toxin. At a high-infection intensity, as a consequence of heavy contaminated air (moulds and mould spores) in warehouses, or when shelves in poor hygienic condition are used, in combination with storage at high humidity, favourable cirumstances are created for production of STC [100]. A second group of stimulating factors are nonorganic phosphates and components of the citric acid cycle (citrates, α-ketoglutarates, succinates, fumarates, malates and acetates) [102].

A 1987 study on STC in products from UK markets failed to detect STC in cheese (0/20) or whole grain maize (0/20) (TLC method's LOD was 20 μg/kg) [103]. In the year 2008 research on STC in cheese was carried out in Latvia and Belgium where STC was detected in 2 out of 21 analyzed cheese samples (different types and age) from local supermarkets. The STC concentrations in the positive samples were 0.52 and 1.23 μg/kg, respectively (LC-MS/MS method's LOD was 0.03 μg/kg) [104].

3 Analytical methods

Two groups of analytical methods for STC in food can be defined: immunochemical ELISA and chromatographic. Competitive ELISA methods have been applied to barley, wheat and other food and feed having LODs of 12–20 μg/kg with average levels found at 20 μg/kg [105–107]. Methods and references for chromatographic detection of STC in different matrices are summarized in Table 1. It shows that extraction methods were commonly based on a mixture of ACN (or methanol) and 4% aqueous potassium chloride, but also $CHCl_3$ and ethyl acetate were used. For defatting of samples *n*-hexane was basically applied while sample cleanup was performed by liquid–liquid extraction (mostly with $CHCl_3$).

Table 1. Summary of chromatographic methods for the detection of STC in foodstuffs, feed, dust, building materials and indoor air

Matrix	Extraction	Defatting and clean up	Separation and detection	LOD (μg/kg)	Recovery (%)	References
Cheese	100 mL 5% NaCl + 200 mL MeOH/ acetone (50/ 50 v/v)	Liquid–liquid extraction with $CHCl_3$ and chloroform–ethyl acetate mixture, SPE on *silicagel* column	TLC-UV with $AlCl_3$	20	87	[108]
Cheese	$CHCl_3$	–	TLC-UV with $AlCl_3$	–	89–97	[109]
Cheese	MeOH/4% KCl (90/10 v/v)	SPE on *Florisil* and *polyamide* columns	TLC-UV with TFA	5	30–80	[23, 97, 110–113]
Cheese	ACN/4% KCl (85/15 v/v)	Cleanup: liquid–liquid extraction with $CHCl_3$ and cupric carbonate column	TLC-UV with Al_2Cl_6	2	86–88	[114, 115]
Cheese	$CHCl_3$	Defatting with *n*-hexane, cleanup: liquid–liquid extraction with $CHCl_3$ and then with ACN	TLC-UV with $AlCl_3$	10	–	[100]
Cheese	ACN/4% KCl (85/15 v/v)	Defatting with *n*-hexane, cleanup: liquid–liquid extraction with $CHCl_3$ and cupric carbonate diatomaceous earth column	TLC-UV	–	85–91	[24]
Cheese	ACN/H_2O (90/ 10 v/v)	Defatting with *n*-hexane. Cleanup: SPE on Strata X SPE column, then evaporation under nitrogen stream, dry residue redissolving in mobile phase	LC-ESI^+-MS/MS	0.03	96–104	[104]
Grains	MeOH/4% KCl (90/10 v/v)	Cleanup: liquid–liquid extraction with $CHCl_3$	TLC-UV with $AlCl_3$	100	60	[67]
Bread, cured ham, salami	ACN/4% KCl (90/10 v/v)	Defatting with *n*-hexane. Cleanup: liquid–liquid extraction with $CHCl_3$, SPE on *silicagel* column	TLC-UV	20	–	[72]
Grains	ACN/H_2O (90/ 10 v/v)	Defatting with *n*-hexane. Cleanup: liquid–liquid extraction with $CHCl_3$, gel permeation chromatography with *polystyrene*	GLC-MS	5	>90	[116]
Rice	Ethylacetate	Evaporation, dissolving in MeOH-20%KCl (4/1 v/v), defatting with *n*-hexane, liquid–liquid extraction with $CHCl_3$, evaporation, dry residue dissolving in acetone, SPE on Sephadex LH-20 column. Evaporation of eluate and dry residue redissolving in acetone	GLC	50	–	[89]
Bread	$CHCl_3$	Evaporation of extract, dry residue re-dissolving in $CHCl_3$ (concentration for five times)	TLC-UV with $AlCl_3$	20	–	[73]
Corn, oats, wheat	ACN/4%KCl (90/10 v/v)	Defatting with *n*-hexane, cleanup: gel permeation chromatography on *silicagel* column	TLC-UV with $AlCl_3$	30	59–138	[117]
Grains, soybeans	MeOH/4%KCl (90/10 v/v)	SPE on *Florisil* column	TLC-UV	50	92–134	[88]
Pistachio nuts	ACN/4% KCl (90/10 v/v)	Defatting with *n*-hexane. Cleanup: liquid–liquid extraction with $CHCl_3$, SPE on *silicagel* column	HPLC-UV	–	–	[91]
Corn, oats	ACN/H_2O (90/ 10 v/v)	Defatting with *n*-hexane, cleanup: gel permeation chromatography on *silicagel* column	HPLC-UV	20	59–74	[118]
Grains, corn, soybeans, feed	ACN/4% KCl (90/10 v/v)	Defatting with isooctane. Cleanup: liquid–liquid extraction with $CHCl_3$	TLC-UV with $AlCl_3$	140	92–95	[119]

Table 1. Continued

Matrix	Extraction	Defatting and clean up	Separation and detection	LOD (μg/kg)	Recovery (%)	References
Maize	MeOH/$CHCl_3$, (50/50 v/v)	Defatting with *n*-hexane. Cleanup by $CHCl_3$-H_2O, and liquid–liquid extraction with $CHCl_3$	TLC-UV with $AlCl_3$	10	–	[120]
Vegetables	Ethylacetate	Defatting with *n*-hexane. Cleanup: liquid–liquid extraction with $CHCl_3$, SPE on *silicagel* column	TLC-UV with TFA anhydride	20	–	[121]
Cocoa beans	ACN/H_3PO_4 (95/5 v/v)	Defatting with *n*-hexane, cleanup: liquid–liquid extraction with $CHCl_3$ silica *Bond-elut* SPE column	HPLC-UV on CN column	13	100–108	[89]
Barley	ACN/4% KCl (90/10 v/v)	Defatting with *n*-hexane, cleanup: liquid–liquid extraction with $CHCl_3$, SPE on *silicagel* column	HPLC-FLD (precolumn derivatization with pyridine and acetic anhydride)	20	31–96	[122]
Corn, cassava flour, rice, dried black beans	MeOH/4%KCl (90/10 v/v)	Cleanup with clarifying agent and *Hyflo Super-Cel* followed by 2 partitions to $CHCl_3$	HPLC-FLD	15–35	98–117	[19]
Maize, bread, cheese	ACN/4% KCl (90/10 v/v) and MeOH/ 4%KCl (90/ 10 v/v)	Defatting with *n*-hexane, cleanup: liquid–liquid extraction with $CHCl_3$	HPLC-$APCI^+$-MS	1.7	118	[83]
				1.9	96	
				2.4	55	
Grains, corn and corn based products etc.	ACN/4% KCl (90/10 v/v)	Defatting with *n*-hexane, cleanup: liquid–liquid extraction with $CHCl_3$, SPE on s*ilicagel* column	HPLC-FLD	3.0	70–110	[93]
Grains, flour, rice	ACN/4% KCl (95/5 v/v)	Defatting with *n*-hexane, cleanup: liquid–liquid extraction with $CHCl_3$, SPE on *phenyl* column	HP-TLC	2.0	80	[60]
				2.0		
				4.0		
Rice	ACN/H_2O (85/15 v/v)	Cleanup: *MycoSep ♯226* column	GC-MS	10[a)]	72	[123]
			LC-MS/MS	4[a)]		
			HPLC-UV	2[a)]		
Grains	ACN/H_2O (84/16 v/v)	SPE on Strata X SPE column, then evaporation under nitrogen and dry residue redissolving in mobile phase	LC-ESI^+-MS/MS	0.15	80–107	[84, 124]
Bread, nuts, rice, cheese, garlic, tomato, apple, lemon, red wine, jam	ACN/H_2O/ CH_3COOH (79/20/1 v/v)	–	LC-ESI-MS/MS	0.4	101–109	[125]
Maize, peanut, pistachio, wheat, raisins, figs	ACN/H_2O (80/20 v/v)	–	LC-MS/MS	10	101–109	[126]
Beer	–	SPE on Strata X SPE column, then concentration of the dry residue and redissolving in mobile phase	HPLC-UV	0.26	81–126	[85]
Feed	$CHCl_3$/4% KCl	Concentration of the chloroform residue	TLC-UV	30	–	[127]
Feed	$CHCl_3$/H_3PO_4 (90/10 v/v)	Gel filtration	TLC-UV	50	–	[128]
Feed	MeOH/4% KCl, 90/10 v/v	Cleanup: SPE on *Florisil* column	TLC-UV with $AlCl_3$	50	92–134	[46]

Table 1. Continued

Matrix	Extraction	Defatting and clean up	Separation and detection	LOD (μg/kg)	Recovery (%)	References
Different foodstuffs	ACN/4% KCl + HCl, 90/10 v/v	Defatting with *n*-hexane, cleanup: liquid–liquid extraction with CH_2Cl_2	TLC-UV	20	80	[129]
Feed, corn, silage	MeOH/4% KCl, 90/10 v/v	Cleanup: SPE on *Florisil* column	TLC-UV with $AlCl_3$	2	–	[94]
Silage	$CHCl_3$	Cleanup: liquid–liquid extraction with H_2O and $CHCl_3$	TLC-UV with $AlCl_3$	–	–	[130]
	ACN/H_2O	–	HPLC-UV	–	–	[62]
	MeOH	–	HPLC-UV	–	–	[63]
Building materials, indoor air, dust	ACN	Filtering through glass fiber filter, dry residue redissolving in MeOH/H_2O (50/50 v/v)	LC-ESI^+-MS/MS	2–4	33	[61]
	Ethyl acetate, then with CH_2Cl_2	Filtering through folded filter, dry residue redissolving in MeOH/H_2O (30/70 v/v)	LC-ESI^+-MS/MS	1.1–1.5 μg/L	61	[64]
	ACN/H_2O + H_3PO_4 (pH 1.5)	Cleanup: SPE on *Chromabond XTR* column	HPLC-UV	100	50–64	[131]

a) Ng on column. Sensitivity of the method in μg/kg not specified.

For sample purification and analyte concentration some authors used SPE on different SPE columns (Florisil, polyamide, cupric carbonate column, polystyrene, silica Bond-elut, Hyflo Super-Cel, phenyl, Chromabond XTR), silicagels being the most popular. Other authors used liquid–liquid extraction coupled to SPE. For the detection and separation (of purified and concentrated sample extracts) of STC the following chromatographic techniques were used: TLC, HPLC, HPLC LC-MS, HPLC LC-MS/MS, GLC-MS and GC-MS. TLC was most frequently used.

TLC methods can be split into two groups: methods with LODs ranging from 2 to 10 μg/kg and methods with LODs from 20 to 140 μg/kg. TLC methods are not very sensitive because STC has no good fluorescence under UV light, but there are some possibilities to increase fluorescence. The first method consists of spraying with an ethanolic $AlCl_3$ solution followed by heating of TLC plates, leading to an Al-complex with the keto- and hydroxyl groups of the STC molecule, resulting in enhancement of the fluorescence intensity for 100 times. In addition, the color of fluorescence changes from brick-red to yellow. In the second method a derivatization step with TFA is included, in which the resulting adduct has a higher fluorescence than the previous after spraying with $AlCl_3$. Until 1979 the more sensitive methods were characterized by LODs of 20 μg/kg. In 1979 TLC methods improved, resulting in a LOD of 10 μg/kg (corn matrix) [120]. In 1980 derivatization with TFA coupled with SPE improved the sensitivity towards a LOD of 5 μg/kg (cheese matrix) [23, 97, 110–113]. In 1985 this result was improved giving a LOD of 2 μg/kg (cheese matrix) [114, 115]. Further, after 9 years a new method with reagent free derivatization was applied for grain analysis giving the same LOD 2–4 μg/kg [122].

TLC methods suffer from insufficient selectivity as matrix components can interfere with STC identification. Sensitivity depends on matrix and TLC methods are usually used as qualitative screening tools. For positive sample confirmation MS methods are required. GC methods are not selective and sensitive enough (LODs 5–50 μg/kg) and require analyte derivatization and MS detection to increase sensitivity and selectivity. This was the reason why Scudamore *et al.* developed a LC-$APCI^+$-MS method in 1996 (LODs 1.9–2.4 μg/kg) [83]. To date modern LC-MS/MS equipment is used for precise and accurate analyte determination, which has following advantages: higher level of selectivity and sensitivity, applicability for fast qualitative screening and for precise quantitative analysis. Therefore, this technique was used for sensitive STC determination in indoor dust, air and mycelium samples [64, 65].

Summarizing the information on STC analytical methods in foodstuffs, authors can conclude that it is required to use LC-MS/MS equipment and to improve sample preparation in order to develop sensitive methods. This was realized in some new methods developed for the determination of STC in different grains and cheese by Veršilovskis *et al.* (LOD 0.03–0.15 μg/kg) [84, 124] and in the multi-mycotoxin method by Sulyok *et al.* (LOD 0.4 μg/kg) [125].

4 Concluding remarks

Summarizing the information presented in this article, it can be concluded that:

(i) STC is produced by different *Aspergillus*, *Penicillium*, *Bipolaris*, *Chaetomium* and *Emiricella* fungal species, so it can be formed under various conditions;

(ii) STC is dangerous to animal health and is a possible human carcinogen (2B);

(iii) STC is frequently reported in different foodstuffs (especially grains, maize, bread and cheese), however there is no systematic monitoring. Also, there is no international legislation on maximum limits of this toxin in foodstuffs;

(iv) Analytical methods for the determination of STC in foodstuffs applied are TLC, GC, GC-MS, HPLC-UV, HPLC-FLD, LC-MS, LC-MS/MS and ELISA. From the available analytical methods in foodstuffs LC-ESI+-MS/MS is the most sensitive;

(v) There is a clear need for systematic research on STC content in different foodstuffs in the EU and other countries in the world.

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