Advances in Basic and Applied Sciences

Green solvents for the extraction of mycotoxins during the analysis of food products: A Mini-review

Mohamed S. Abdelfattah[a,b*]

[a] *Chemistry Department, Faculty of Science, Helwan University, Egypt.*
[b] *Natural Products Research Unit, Chemistry Department, Faculty of Science, Helwan University, Cairo 11795, Egypt*

ARTICLE INFO

ABSTRACT

Implementing green chemistry concepts in all areas of science for eco-friendly and sustainable scientific research is necessary. A promising area in which green chemistry principles may have a substantial impact on the quality and efficiency of natural products is the extraction of mycotoxins. These compounds are poisonous to humans and are frequently found in food and feed. In this literature review, we present the traditional extraction methods of mycotoxins during food product analysis using toxic organic solvents and updated technical information about green solvents such as deep-eutectic solvents, supercritical CO_2, and pressurized hot water. Within this review, the extraction of various types of mycotoxins has been discussed, with an emphasis on the factors that influence their extraction, such as the molar ratio of the extracting solvent, the extraction temperature, co-solvents, the extraction duration, and the recovery percentage.

1. Introduction

Mycotoxins are a type of naturally occurring toxic secondary metabolites that can be harmful to human health and are known to cause cancer, hepatotoxicity, immunosuppression, pneumonia, asthma, and exhaustion [1–3]. They also have various effects on the processes of metabolism within the body, such as inhibiting protein synthesis, disrupting hormonal balance, causing a variety of reproductive system diseases, and generally harming the health of the host [1,4]. Mycotoxins are produced by a wide variety of fungus species like *Aspergillus*, *Penicillium*, *Trichoderma*, and *Fusarium* when environmental conditions are ideal for fungal growth [5]. These compounds can be found in a variety of foods and feeds, including cereals, legumes, seeds, and milk, as well as vegetables and fruits [6,7]. High temperatures, moisture levels, poor hygienic conditions, and contamination during storage and transit all enhance mycotoxin growth in these products [8]. Several natural mycotoxins such as

[*] **Corresponding author E-mail**: mabdelfattah@science.helwan.edu.eg

aflatoxins, zearalenone, ochratoxin A, alternariol, and patulin are reported in literature (Fig. 1) [9]. Aflatoxins are characterized by a difuranocoumarin skeleton and are formed by several fungal strains like *Aspergillus parasiticus* and *Aspergillus flavus* via the polyketide biosynthetic pathway. The most famous aflatoxins are aflatoxins B1, B2, G1, and G2, which can be differentiated by observing their UV fluorescence (green or blue) and comparing their *Rf* values on thin-layer chromatography [10,11]. Aflatoxin M is also a hydroxylated derivative of aflatoxin B1, which is commonly found in milk and milk-based infant formula [12]. On the other hand, zearalenone, which is a nonsteroidal estrogenic mycotoxin, is produced by various *Fusarium* species like *Fusarium graminearum*, *F. culmorum*, *F. equiseti*, and *F. crookwellense* and transformed into α-zearalenol and β-zearalenol in animals [13]. These compounds can be detected under UV light and by HPLC/MS/MS [14,15]. Ochratoxin A is a dihydrocoumarins family pentaketide derivative coupled to β-phenylalanine generated by several *Aspergillus* and *Penicillium* species. It has been identified in food products, most notably contaminated grain, coffee, dried grapes, and breast milk [16]. The UV-visible, fluorescence, NMR, and MS detection methods are used to characterize ochratoxin A [17]. Alternariol and alternariol monomethyl ether are a benzochromenone-type mycotoxin produced by *Alternaria* fungi and found in a variety of cereals as well as diseased fruits such as mandarins, oranges, lemons, melons, apples, and berries [18]. Patulin is a harmful unsaturated heterocyclic lactone derivative that is biosynthesized by a variety of toxigenic *Aspergillus* species via a polyketide pathway. *Penicillium expansum* is the principal contributor to patulin production in rotting apples and has been identified in commercial apple juice and other apple products [19]. To identify and quantify patulin, liquid chromatography (LC) with a UV detector and LC/MS/MS was used [20].

The conventional extraction methods applied to extract the above-mentioned mycotoxins from food samples are carried out using toxic organic solvents that are not safe for both environment and human health [21]. This review presents a brief discussion about various type of mycotoxins and the current understanding of alternative green solvents (i.e. deep-eutectic solvents, supercritical CO_2, and pressurized hot water) used in laboratories, with an emphasis on extraction parameters, advanced protocols, and the development of safe methods.

Methodology of the literature review

In this review, the author explored the relevant literature using multiple online databases, including SciFinder, Scopus, PubMed, Google Scholar, Web of Science, and Science Direct. He looked for "green solvents, mycotoxins, deep-eutectic solvents, supercritical CO_2 extraction, and pressurized hot water. Reports released in the past 10 years were more focused than other research works, and the published papers with only abstracts, unfinished works, conference proceedings, and publications in languages other than English were disregarded.

Conventional extraction methods for mycotoxins

Different traditional methods for extraction of bioactive secondary metabolites [22–27], including mycotoxins from different nutrient materials[28,29], using organic solvent extraction (OSE) have been reported. In the OSE extraction methods analytes are separated according to their relative solubility in two immiscible liquids. Solvent extraction typically employs methanol, acetonitrile, chloroform, ethyl acetate, isooctane, ethanol, and dichloromethane [30]. Patterson and Roberts' multi-mycotoxin extraction method is the most extensively utilized solvent extraction method for aflatoxins and used acetonitrile, isooctane, potassium chloride, dichloromethane, and sulfuric acid in this approach [31,32]. It has gained popularity because it selectively extracts many mycotoxins in a single extraction. In order to use SE, however, considerable amounts of organic solvents need to be consumed, which might be harmful to the environment. In addition, solvent extraction is a time-consuming operation that can last up to 24 hours or more. In addition, high-purity solvents are expensive, and there are sometimes other expenses associated with properly disposing of wastes after usage [29]. In addition to liquid-liquid extraction, solid-phase extraction (SPE) is frequently employed for aflatoxins [33]. Analytes are separated using a liquid mobile phase and a solid stationary phase in a cartridge. Ethyl (C2), octyl (C8), octadecyl (C18), cyanopropyl (CN), aminopropyl (NH), and an ion exchange phase are all examples of materials employed at the solid adsorbent phase. Although SPE techniques are very easy, have higher specificity, and need little amounts of solvent, they are also quite expensive, and antibodies for some mycotoxins and products are not accessible. Alternariol and alternariol monomethyl ether had been extracted with acetonitrile or ethyl acetate using solid-liquid extraction method, followed by QuEChERS and dilution-direct injection [34]. Patulin was also extracted from various fruit juices (i.e., apples, pineapples, grapes, pears, etc,) using ethyl acetate as a solvent [35,36]. Solid-phase extraction (SPE) is another extraction method, for patulin, in which the juice sample is mixed with a solvent (such as acetonitrile) and eluted through a solid phase. This technique was found to be suitable for isolating, concentrating, and purifying patulin [37].

Deep eutectic solvents

Deep eutectic solvents (DESs) are a new class of green solvents that have gotten a lot of attention from scientists in recent decades [38–41]. These DESs can be created simply by combining two eco-friendly components, hydrogen bond donors (HBDs) and acceptors (HBAs), which are capable of forming a eutectic mixture with a low melting point. Choline chloride (ChCl) is a common material used in the manufacture of these DES and is characterized by its low cost, biodegradability, and absence of toxicity [42]. It can rapidly form a DES when combined with safe hydrogen bond donors such as urea, carboxylic acids (e.g., oxalic, citric, succinic, or amino acids), or polyalcohol like glycerol and carbohydrates

[43]. The most common used deep eutectic solvents (DESs) are shown in Fig. 2.
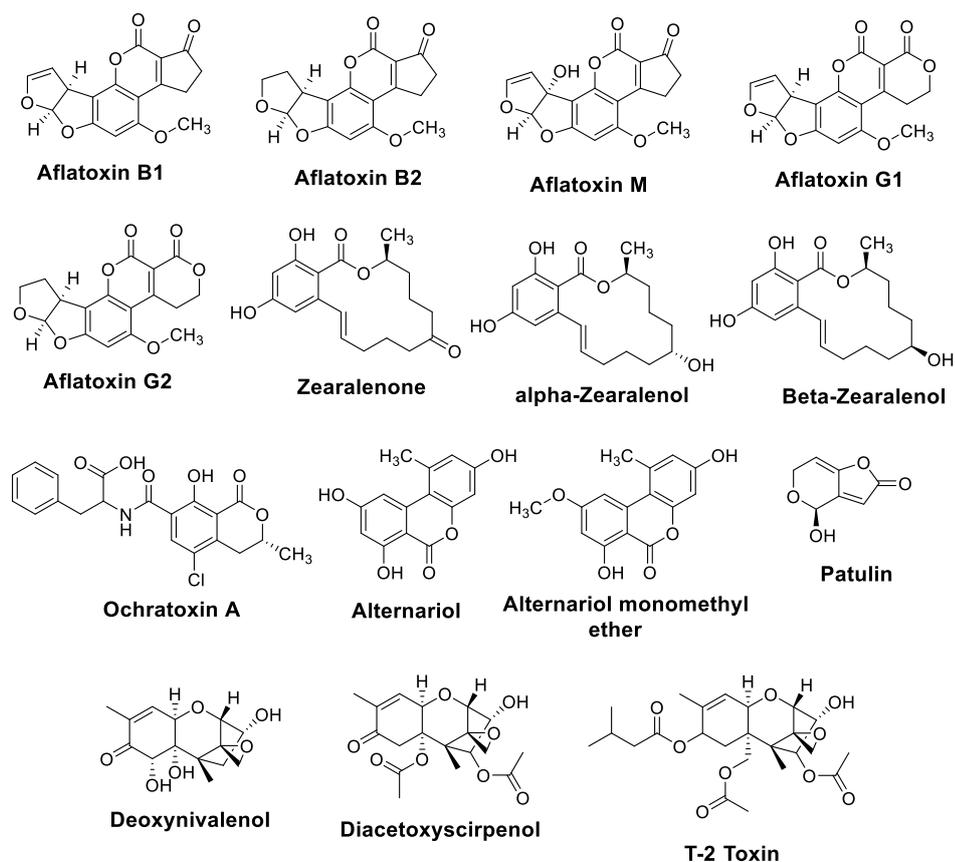
Fig. 1: Chemical structures of different mycotoxins
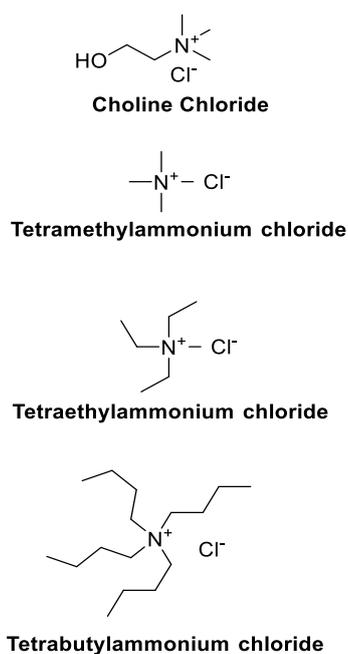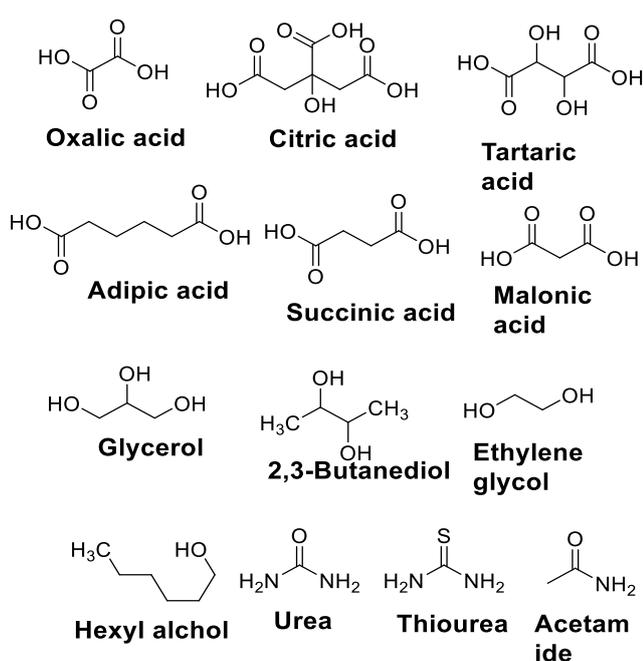
Fig. 2: Most common hydrogen bond acceptors and hydrogen bond donors used for the preparation of DESs

DESs have applications in the extraction of bioactive molecules such as phenolic compounds, alkaloids, terpenoids, carbohydrates, fatty acids, natural pigments, tannins, and nutraceutical components [44,45]. He *et al.* have been extrcted aflatoxins B1, B2, G1, and G2 from different oil samples (i.e., corn oil, soybean oil, peanut oil, and rapeseed oil) using a series of DESs consisting of choline chloride as a hydrogen bond-acceptor and six acidic hydrogen-bond-donor (i.e., oxalic acid, lactic acid, malonic acid, levulinic acid, trifluoroacetic acid, and acetic acids) in different molar ratios (Table 1). The extraction efficiency of aflatoxins depended on the type of hydrogen-bond donor, extraction time, extraction temperature, and volume of DESs. The authors reported the best extraction yield of each aflatoxin was reached when malonic acid was used as HBD. The optimal extraction was obtained using ultrasonic for 50 minutes at a temperature of 55°C and ChCl/malonic acid molar ratio of 1:2. The technique exhibited better extraction efficiency than the usual derivatization technique with trifluoroacetic acid. In 2020, the same aflatoxins B1, B2, G1, and G2 were extracted and determined in ten rice samples using DESs without any derivatization [46]. Six DESs based on a combination of three hydrogen bond-acceptors (i.e., tetramethylammonium chloride, choline chloride, and betaine) and malonic acid as hydrogen-bond-donor were investigated. The maximum recovery (78.93-113.64%) were obtained with tetramethylammonium chloride/ malonic acid/water (1:2:1 mole ratio) with a liquid/solid ratio of 3 mL/g. The extraction was assisted with vortex and ultrasonic for 50 minutes at 55°C. Wu et *al.* evaluated the potential of deep eutectic solvent-based matrix solid-phase dispersion (DES-MSPD) to extract aflatoxins B1, B2, G1, and G2 from millet, peanut, and heepseed [47]. The authors used tetrabutylammonium chloride (TBAC) as a hydrogen bond-acceptor and hexyl alcohol, dodecyl alcohol as well as hexanoic acid as hydrogen-bond-donor. Tetrabutylammonium chloride (TBAC)/hexyl alcohol DES showed the best recoveries for the target aflatoxins B1, B2, G1, and G2 with recoveries 97.87% ~ 98.07%. Additionally, they used silica gel (Al$_2$O$_3$) and diatomite as dispersants in their MSPD process. They found that silica gel was the best dispersant for the extraction of aflatoxins from the millet sample, whereas the diatomite was the top for the peanut and hempseed specimens.

In 2019, patulin was extracted from different natural juices (i.e., apple, orange, peach, apricot,.etc) using alcohol-based deep eutectic solvents with ultrasound-assisted emulsification liquid-phase microextraction (ELPME) [37] (Table 1). The technique depended on forming a complex between patulin and Mg(II) in the presence of tris buffer. The authors were successfully extracted the formed complex using seven deep eutectic solvents. The extraction efficiency was enhanced using 500 µL of TBACl/2,3-butanediol, 75 µmol L^{-1} of Mg(II) as a complexing agent at pH 8.0, and assistance of ultrasound for 12 minutes. The best recovery percentage from different juices (90.2% to 106.9%) was obtained using tetrabutylammonium chloride (TBACl) and 2,3-butanediol in the molar ratio 1:2. Ochratoxin A was extracted from four cereal-based products (i.e., wheat, bread crumbs, biscuits, and bran) using two eutectic mixtures of choline chloride/glycerol (1:2) and choline chloride/urea (1:2) combined with water [48]. The best conditions for the extraction were found after 60 min of extraction with choline chloride/urea (1:2) and 40% (w/w) of water at room temperature. In these conditions, the mean recoveries of ochratoxin A from durum wheat, breadcrumbs, biscuits, and bread were 70%, 88%, 75%, and 42%, respectively. The authors found that increasing the extraction temperature to 40°C with sonication didn't increase the recovery percentages.

Table 1: Application of DESs on the extraction of mycotoxins from some food products

Extraction solvent	Molar ratio	Type of mycotoxin	Food products	Extraction method [a]	Recovery (%)	Ref.
Choline chloride/ malonic acid	1:2	Aflatoxins B1, B2, G1, and G2	Corn oil, soybean oil, peanut oil, and rapeseed oil	UA/55 °C	72.1–113.5	[46]
Tetramethy lammonium chloride/malonic acid	1:2	Aflatoxins B1, B2, G1, and G2	Rice samples	Vortex-UA/55 °C	78.9–113.6	[46]
Tetrabutyla mmonium chloride (TBAC)/hexyl alcohol	1:1	Aflatoxins B1, B2, G1, and G2	Millet, peanut, and heepseed	MSPD	93.7-97.9	[46]
Tetrabutyla mmonium chloride/2,3-butanediol	1:2	Patulin	Apple juice, orange juice, peach juice, apricot juice, grape juice, kiwi juice, cherry juice and mango juice	UA-ELPME/ room temperature	90.2-106.9	[37]
Choline chloride/ urea	1:2	Ochratoxin A	Wheat, breadcrumbs, biscuits and bran	Shaking/ room temperature	7.00-89.0	[48]

[a] MSPD, matrix solid phase dispersion; UA, ultrasound-assisted; UA-ELPME, ultrasound-assisted emulsification liquid phase microextraction.

Supercritical CO₂ as a green solvent

Supercritical CO_2 extraction is a fascinating approach that employs carbon dioxide (CO_2) as a non-toxic solvent, which is produced as a byproduct of several industrial processes and is recoverable at the end of the extraction methods [49–51]. The basic principle of this technology is achieving a homogenous phase after the evaporation of the vapour phase and the separation of liquid beyond the critical point. There are two critical points in this technology: critical temperature and critical pressure. Critical temperature is the temperature at which a gas cannot become liquid as long as there is no extra pressure; and, critical pressure is the minimum amount of pressure needed to liquefy a gas at its critical temperature. This supercritical phase facilitates the transformation of the fluid into a supersolvent, thereby increasing the extraction's efficacy [52,53]. Supercritical CO_2 extraction has several advantages over traditional solvent extraction methods, such as high extraction efficiency due to its great diffusivity, high density, which vary with temperature, as well as its low viscosity, which make it ideal for the quick, selective extraction and fractionation of compounds [54,55]. Moreover, high-quality extracts can be easily precipitated after a reduction in pressure without the use of solvents, as a result of the limited thermal degradation, short extraction periods, and high selectivity [56,57]. A number of procedures have been recently created owing to these benefits, such as the extraction of lipids from olive mill wastewater [58] and microalgae [59], and seed oils from pomegranate and alfalfa [60]. In case of mycotoxins (Table 2), Zougagh and Ríos extracted several macrocyclic lactone mycotoxins (i.e., zearalenone, α- and β-zearalenols) from maize flour by supercritical CO_2 extraction [61]. The authors carried out the extraction at different temperatures (50, 60, 70, and 80ºC) at constant pressure (25 MPa). The highest recovery percent (90–92%) was achieved at a flow-rate of 1 ml/min CO_2 at 80ºC, for 10 minutes of static extraction followed by 30 minutes of the dynamic model. The authors used 9.1% of methanol as a co-solvent to get the highest extraction yield. In this technique, a Florisil cartridge coupled to the extractor vessel was used to clean-up the analytes and detected the compounds by electrochemical and HPLC systems. The same author with his co-workers developed and validated a computerized program to gain automatically and process the same mycotoxins data from the electrochemical detector and get the results [62]. They carried out the extraction using the same method and conditions, except the flow rate (1.3 ml/min) and pressure (30 MPa).

From the Zizyphi Fructus, a common fruit in traditional Chinese medicine, aflatoxins B1, B2, G1, and G2 were extracted using the supercritical fluid CO_2 extraction [63]. A high viscosity characterizes these fruits, after grinding, due to the absorption of moisture from the air. Therefore, sand should be added (1:1) during pulverization to reduce humidity's effect on the extraction efficiency. The best conditions for higher recovery percent (98-105%) were obtained using 20 ml of CO_2 at 50ºC, a pressure of 41.4 MPa for 15 min of dynamic extraction time, and 15 min of static extraction time. The resultant extracts were collected in ethyl acetate extract to reduce the adverse influences on the environment. In 1998, different *Fusarium* mycotoxins (i.e., 3-acetyldeoxynivlenol, 15-acetyldeoxynivlenol, deoxynivlenol), and derivatives of type B trichothecenes (i.e., fusarenone X and nivalenol) were extracted from the naturally infected and spiked wheat flour with supercritical CO_2 with modifiers [64]. The reported conditions for a higher recovery percentage (53%) were the static mode for 30 minutes followed by a dynamic mode for 15 minutes with 2 ml/min flow rate of CO_2 and 3% of methanol as a modifier. The optimum temperature and pressure for the extraction method were 55ºC, and 31.8 MPa, respectively. The analytes were passed through silica gel as a solid-phase trap. In 1997, the efficiency of supercritical CO_2 to extract aflatoxin M1 from beef liver was investigated [65]. The best recovery was achieved with a CO_2 flow rate of 5 liters/min at a pressure of 58.6 MPa for 30 minutes in dynamic mode at 80ºC. The authors used acetonitrile/methanol (2:1, 3.3 vol%) as organic modifiers to improve the target analyte's recovery. This technique can be used instead of the conventional extraction method that uses methylene chloride as an organic solvent. Huopalahti *et al.* extracted the 12,13-epoxytrichothecene mycotoxins (i.e., deoxynivalenol, deacetoxyscirpenol, and T-2 toxin) from the yellow cornmeal and rolled oats by using supercritical fluid extraction with CO_2 and adding 5% of methanol as a co-solvent [66]. The extraction was optimized with the following conditions: the pressure of 55.7 MPa, a flow rate of CO_2 1.2 ml/min at a temperature of 60ºC. The process gave a recovery percent up to 95% for deoxynivalenol and 85% for deacetoxyscirpenol and T-2 toxin. The target analytes were collected in methanol and defatted with hexane. Holcomb *et al* developed a supercritical CO_2 method by adding methanol as a co-solvent to extract aflatoxins B1, B2, G1, and G2 from corn [67]. The extraction was effective at the static conditions at 65ºC, 51.7 MPa for 15 minutes, followed by a dynamic model for 10 minutes with 20 ml of liquid CO_2. The extract was collected in chloroform and purified over Sep-Pak Florisil cartridge, and the recovery percent reached 77.3, 82.9, 75.4, and 80.3% for aflatoxins B1, B2, G1, and G2, respectively. This technique was effective against the corn contaminated with natural aflatoxins, specifically for aflatoxin G1.

Wu *et al.* extracted aflatoxins B1, B2, G1, and G2 from peanut kernels contaminated with *Aspergillus parasiticus* using supercritical CO_2 extraction plus 60% methanol [68]. The extraction process's optimum conditions were 50°C for 15 minutes and pressured up to 20.7 MPa. The authors found that the best recovery percent (97.7%) was obtained by adding 60% of methanol as a co-solvent and adjusting the methanol/ peanut meal ratio to 6 (vol/wt). Taylor et al. proposed 34.5 MPa and 80°C as proper conditions to accomplish supercritical fluid extraction of aflatoxin B1 from commercial corn samples [69]. The authors studied

Table 2: Application of supercritical CO_2 extraction in some food products

Extraction solvents	Type of mycotoxins	Food products	Extraction method[a]	Temperature (°C)/ Pressure (MPa)	Extraction time in min (static + dynamic)	Flow rate of CO_2 (ml/min)	Recovery (%)	Ref.
CO_2 (+ 9.1% methanol)	Zearalenone, α-Zearalenol and β-zearalenol	Maize flour	SPE	80/25	10+30	1	90–92	[61]
CO_2 (+ 10% methanol)	Zearalenone,, α-Zearalenol and β-zearalenol	Maize flour	SPE	80/30	30+10	1.3	90–92	[61]
CO_2	Aflatoxins B1, B2, G1, and G2	Zizyphi Fructus	Solvent (ethyl acetate)	50/41.4	15 + 15	-	98-105	[63]
CO_2 (+ 3% methanol)	Deoxynivlenol, 3-acetyldeoxynivlenol, 15-acetyldeoxy-nivlenol, fusarenone X and nivalenol	Wheat	SPE	55/31.8	30 +15	2	53	[64]
CO_2 (+ acetonitrile: methanol 2:1)	Aflatoxin M1	Beef liver	SPE	80/58.6	0+ 30	5000	0.36*	[65]
CO_2 (+ 5% methanol)	Deoxynivalenol, deacetoxyscirpenol, and T-2 toxin	Yellow corn meal and rolled oats	Bubbling in methanol followed by defatting with hexane and evaporation	60/55.7	-	1.2	85-95	[66]
CO_2 (+methanol)	Aflatoxins B1, B2, G1, and G2	Corn	SPE	65/51.7	10 +15	-	77.3–80.3	[66]
CO_2 (+ 60% methanol)	Aflatoxins B1, B2, G1, and G2	Peanut kernels	IAC	50/20.7	15	-	97.6	[68]
CO_2 (+ acetonitrile: methanol 2:1)	Aflatoxin B1	Corn	SPE	80/34.5	-	-	90	[69]
CO_2 (+ 5.6% methanol)	Aflatoxin B1	Peanut meals, animal feed	SFE	40/83.1	150	270	>70	[70]

[a] SPE, solid-phase extraction; IAC, Immunoaffinity column.
*Recovery is expressed in ppb

Table 3: Application of pressurized hot water extraction in some food products

Extraction solvents	Type of mycotoxins	Matrix	Extraction method[a]	Temperature (°C)/ Pressure (MPa)	Extraction time (min)	Flow rate of (ml/min)	Recovery (%)	Ref.
Alkaline aqueous solution	Aflatoxins B1, B2, G1, and G2	TCMs	d-SPE	100/-	10	-	72.7-114.5	[80]
PHW (+ 45% methanol)	Multi-mycotoxins (i.e., Aflatoxins B1, B2, G1, and G2; alternariol monomethyl ether; fumonisins B1, B2, and B3; ochratoxins A and B; sterigmatocystin; T-2 toxin; zearalenone; α-zearalenol; and β-zearalenol)	Maize Sorghum Millet		162/6.89		5	14–129	[78]
PHW (+ 40% methanol)	Aflatoxins B1	Maize	-	100/-	-	-	116	[79]

PHW, pressurized hot water; TCM, Traditional Chinese medicines; d-SPE, dispersive solid phase extraction.

different parameters like pressures (13.8-103.4 MPa), temperatures (40-80°C), amount of supercritical carbon dioxide (SC-(202) (up to 0.5 liters of CO_2), and co-solvents to enhance the extraction method. The optimum organic modifier was 15% of acetonitrile/methanol (2:1). The resulting extract was trapped in different solvents, including chloroform, which is regarded as a harmful solvent. This technique gave more than 90% aflatoxin B1 recovery when compared to the traditional solvent extraction method. Aflatoxin B1 was also extracted from peanut meals and animal feed using supercritical fluid extraction using CO_2 plus 5.6% of methanol as a modifier [70]. Different temperatures and pressures were applied to increase the extraction method's selectivity and get a higher recovery percent. The optimum operating

conditions were 40ºC at a pressure of 83.1 MPa for 150 minutes and a CO_2 flow rate of 270 ml/min.

Pressurized hot water as a green solvent

Pressurized hot water extraction (PHWE) or subcritical water extraction (SWE) is an eco-friendly extraction technology in which water becomes subcritical when heated between its typical boiling point of 100°C and its critical point of 347°C under high pressure (i.e., 221.2 bar) [55,71,72]. Under such conditions, water remains in liquid form and is considered a good substitute for organic solvents. The polarity of PHW can be controlled by adjusting the temperature and pressure, where the dielectric constant is the parameter utilized to calculate the polarity of water at subcritical conditions [73]. The advantage of PHWE over conventional methods is that it can extract compounds with different polarities in shorter extraction times and higher extraction yields. It was used to extract polyphenols from *T. chebula* fruits, pectins found in citrus peel and apple pomace, flavanones from orange peels [74], tannins from the bark of Norway spruce and Scots pine [75], and bioactive compounds from okra seeds [76] and garlic [77].

Gbashi and his co-workers started to exctract aflatoxin B1 (Table 3) from maize using pressurized hot water extraction (PHWE) [78,79]. The authors carried out the extraction at different temperatures and various percentages of methanol (0%, 20%, 40%, and 60%) as a cosolvent. They documented that the optimal conditions for the extraction were 100ºC with the addition of 40% methanol. The maximum recovery rate was 116%. The same research group extracted multi-mycotoxins from several maize samples in a single step using pressurized hot water extraction (PHWE) with ethanol (EtOH) as a cosolvent [78,79]. They optimized temperature and solvent composition to be 162°C and 45%, respectively, and achieved recoveries (14-129%) higher than other conventional mycotoxin extraction methods. Wang *et al*. investigated the extraction of aflatoxins B1, B2, G1, and G2 from several types of traditional Chinese medicines (i.e., rhizomes, roots, fruits, seeds, flowers, grasses, and leaves) using only water at pH of 13, followed by dispersive solid-phase extraction at room temperature for 10 minutes [80]. The method was based on the cleavage of the lactone ring of aflatoxins in a strong alkaline media. Then, absorbed the formed negatively charged substituted coumaric acid on the positively charged mixed-mode anion exchange (MAX) followed by elution with acetonitrile/trifluoroacetic acid. The authors studied the extraction efficiency at different pH values starting from 9 to 14 and found the efficiency was enhanced from 9 to 13 and declined from pH 13 to 14. Therefore, pH 13 was selected as the optimum value for the extraction methodology. The recovery rate was found to be 72.7-114.5% for the 15 kinds of traditional Chinese medicines (TCMs).

Conclusions

This review summarizes the recent trends in using different green solvents to extract different types of mycotoxins during the analysis of food products. It was found that both deep-eutectic solvents and supercritical CO_2 have great potential for multi-mycotoxin extraction with a higher recovery percent, than pressurized hot water. This review will be useful to researchers as well as industries involved in mycotoxin research to choose eco-friendly method with the appropriate green solvents for increasing the quality and efficacy of analysis.

References

1. Awuchi CG, Ondari EN, Nwozo S, et al. Mycotoxins' Toxicological Mechanisms Involving Humans, Livestock and Their Associated Health Concerns: A Review. Toxins (Basel). 2022;14(3):167. doi:10.3390/toxins14030167

2. Roy S, Shaw D, Sarkar T, Choudhury L. Mycotoxins in fermented foods : A comprehensive review 2 . Contamination of fermented foods with mycotoxins. 2023;7:1897-1917.

3. Wu F. Mycotoxin risks are lower in biotech corn. *Curr Opin Biotechnol*. 2022;78:102792. doi:10.1016/j.copbio.2022.102792

4. Bulgaru CV, Marin DE, Pistol GC, Taranu I. Zearalenone and the Immune Response. *Toxins (Basel)*. 2021;13(4). doi:10.3390/TOXINS13040248

5. González-Jartín JM, Ferreiroa V, Rodríguez-Cañás I, et al. Occurrence of mycotoxins and mycotoxigenic fungi in silage from the north of Portugal at feed-out. *Int J Food Microbiol*. 2022;365:109556. doi:10.1016/j.ijfoodmicro.2022.109556

6. Pushparaj K, Meyyazhagan A, Pappuswamy M, Mousavi Khaneghah A, Liu WC, Balasubramanian B. Occurrence, identification, and decontamination of potential mycotoxins in fruits and fruit by-products. *Food Front*. 2023;4(1):32-46. doi:10.1002/fft2.198

7. Fernández-Cruz ML, Mansilla ML, Tadeo JL. Mycotoxins in fruits and their processed products: Analysis, occurrence and health implications. *J Adv Res*. 2010;1(2):113-122. doi:10.1016/j.jare.2010.03.002

8. Shi H, Li S, Bai Y, Prates LL, Lei Y, Yu P. Mycotoxin contamination of food and feed in China: Occurrence, detection techniques, toxicological effects and advances in mitigation technologies. *Food Control*. 2018;91:202-215. doi:10.1016/j.foodcont.2018.03.036

9. Rashad YM, Baka ZAM, Moussa TAA. Mycotoxins and Their Producers: Diversity, Side Effects and Control. *Plant Mycobiome*. 2023:1-27. doi:10.1007/978-3-031-28307-9_1

10. Wang X, Wang T, Nepovimova E, Long M, Wu W, Kuca K. Progress on the detoxification of aflatoxin B1 using natural anti-oxidants. *Food Chem Toxicol*. 2022;169:113417. doi:10.1016/j.fct.2022.113417

11. Rocha AR, Cardoso MS, Souza Júnior JA, Gomes Júnior EA, Maciel LF, Menezes-Filho JA. Occurrence

of aflatoxins B1, B2, G1, and G2 in beers produced in Brazil and their carcinogenic risk evaluation. *Food Control*. 2023;145:109348.

12. Jafari K, Fathabad AE, Fakhri Y, et al. Aflatoxin M1 in traditional and industrial pasteurized milk samples from Tiran County, Isfahan Province: A probabilistic health risk assessment. *Ital J Food Sci*. 2021;33(1):103-116. doi:10.15586/ijfs.v33iSP1.2054

13. Lu Q, Luo JY, Ruan HN, Wang CJ, Yang MH. Structure-toxicity relationships, toxicity mechanisms and health risk assessment of food-borne modified deoxynivalenol and zearalenone: A comprehensive review. *Sci Total Environ*. 2022;806:151192. doi:10.1016/j.scitotenv.2021.151192

14. Zhang Y, Liu D, Peng J, Cui Y, Shi Y, He H. Magnetic hyperbranched molecularly imprinted polymers for selective enrichment and determination of zearalenone in wheat proceeded by HPLC-DAD analysis. *Talanta*. 2020;209:120555. doi:10.1016/j.talanta.2019.120555

15. Songsermsakul P, Sontag G, Cichna-Markl M, Zentek J, Razzazi-Fazeli E. Determination of zearalenone and its metabolites in urine, plasma and faeces of horses by HPLC-APCI-MS. *J Chromatogr B Anal Technol Biomed Life Sci*. 2006;843(2):252-261. doi:10.1016/j.jchromb.2006.06.012

16. Mwabulili F, Xie Y, Li Q, Sun S, Yang Y, Ma W. Research progress of ochratoxin a bio-detoxification. *Toxicon*. 2023;222:107005. doi:10.1016/j.toxicon.2022.107005

17. Zhang W, Wang J, Dou J, et al. A novel investigated method for decoupling adsorption and degradation effect on AFB1 based on isotope tracing and NMR analysis. *Food Chem*. 2023;405:134978. doi:10.1016/j.foodchem.2022.134978

18. Islam MT, Martorell M, González-Contreras C, et al. An updated overview of anticancer effects of alternariol and its derivatives: underlying molecular mechanisms. *Front Pharmacol*. 2023;14. doi:10.3389/FPHAR.2023.1099380

19. Bacha SAS, Li Y, Nie J, Xu G, Han L, Farooq S. Comprehensive review on patulin and Alternaria toxins in fruit and derived products. *Front Plant Sci*. 2023;14. doi:10.3389/fpls.2023.1139757

20. Sadok I, Stachniuk A, Staniszewska M. Developments in the Monitoring of Patulin in Fruits Using Liquid Chromatography: an Overview. *Food Anal Methods*. 2019;12(1):76-93. doi:10.1007/s12161-018-1340-9

21. Zhang L, Dou XW, Zhang C, Logrieco AF, Yang MH. A review of current methods for analysis of mycotoxins in herbal medicines. *Toxins (Basel)*. 2018;10(2). doi:10.3390/toxins10020065

22. Yixizhuoma, Ishikawa N, Abdelfattah MS, Ishibashi M. Elmenols C-H, new angucycline derivatives isolated from a culture of Streptomyces sp. IFM 11490. *J Antibiot (Tokyo)*. 2017;70(5):601-606.

doi:10.1038/ja.2016.158

23. Kim MC, Cullum R, Hebishy AMS, et al. Mersaquinone, a new tetracene derivative from the marine-derived streptomyces sp. Eg1 exhibiting activity against methicillin-resistant staphylococcus aureus (mrsa). *Antibiotics*. 2020;9(5). doi:10.3390/antibiotics9050252

24. Abdelfattah MS, Toume K, Ishibashi M. Izumiphenazine D, a new phenazoquinoline N-oxide from Streptomyces sp. IFM 11204. *Chem Pharm Bull*. 2011;59(4):508-510. doi:10.1248/cpb.59.508

25. Abdelfattah MS, Elmallah MIY, Faraag AHI, Hebishy AMS, Ali NH. Heliomycin and tetracinomycin D: anthraquinone derivatives with histone deacetylase inhibitory activity from marine sponge-associated Streptomyces sp. SP9. *3 Biotech*. 2018;8(6). doi:10.1007/s13205-018-1304-1

26. Abdelfattah M, Maskey RP, Asolkar RN, Grün-Wollny I, Laatsch H. Seitomycin: Isolation, structure elucidation and biological activity of a new angucycline antibiotic from a terrestrial streptomycete. *J Antibiot (Tokyo)*. 2003;56(6):539-542. doi:10.7164/antibiotics.56.539

27. Abdelfattah MS, Arai MA, Ishibashi M. Bioactive secondary metabolites with unique aromatic and heterocyclic structures obtained from terrestrial actinomycetes species. *Chem Pharm Bull*. 2016;64(7):668-675. doi:10.1248/cpb.c16-00038

28. Cigić IK, Prosen H. An Overview of Conventional and Emerging Analytical Methods for the Determination of Mycotoxins. *Int J Mol Sci*. 2009;10(1):62. doi:10.3390/IJMS10010062

29. Janik E, Niemcewicz M, Podogrocki M, et al. The existing methods and novel approaches in mycotoxins' detection. *Molecules*. 2021;26(13). doi:10.3390/molecules26133981

30. Turner NW, Bramhmbhatt H, Szabo-Vezse M, Poma A, Coker R, Piletsky SA. Analytical methods for determination of mycotoxins: An update (2009-2014). *Anal Chim Acta*. 2015;901:12-33. doi:10.1016/J.ACA.2015.10.013

31. Chemat F, Vian MA, Ravi HK, et al. Review of alternative solvents for green extraction of food and natural products: Panorama, principles, applications and prospects. *Molecules*. 2019;24(16). doi:10.3390/molecules24163007

32. Steyn PS. Mljltimycotoxin analysis. *Pure Appl Chem*. 1981;53(4):891-902. doi:10.1351/pac198153040891

33. Z. Magoke G, G. Alders R, Krockenberger M, L. Bryden W. Aflatoxin and Mycotoxin Analysis: An Overview Including Options for Resource-limited Settings. In: *Aflatoxins - Occurrence, Detection and Novel Detoxification Strategies*. IntechOpen; 2022. doi:10.5772/intechopen.106834

34. Escrivá L, Oueslati S, Font G, Manyes L. Alternaria

Mycotoxins in Food and Feed: An Overview. *J Food Qual*. 2017;2017. doi:10.1155/2017/1569748

35. Rosa da Silva C, Tonial Simões C, Kobs Vidal J, Reghelin MA, Araújo de Almeida CA, Mallmann CA. Development and validation of an extraction method using liquid chromatography-tandem mass spectrometry to determine patulin in apple juice. *Food Chem*. 2022;366:130654. doi:10.1016/j.foodchem.2021.130654

36. Mahmoud AR, Hebishy ; A M S, Essawy EA, Abdelfattah MS. Quantification of the Mycotoxin Patulin and Hydroxymethylfurfural in Some Juice in the Egyptian Markets. *Egypt J Appl Sci*. 2022;37(1):1-10. doi:10.21608/ejas.2022.228054

37. Notardonato I, Gianfagna S, Castoria R, et al. Critical review of the analytical methods for determining the mycotoxin patulin in food matrices. *Rev Anal Chem*. 2021;40(1):144-160. doi:10.1515/REVAC-2021-0131/ASSET/GRAPHIC/J_REVAC-2021-0131_FIG_002.JPG

38. Florindo C, McIntosh AJS, Welton T, Branco LC, Marrucho IM. A closer look into deep eutectic solvents: Exploring intermolecular interactions using solvatochromic probes. *Phys Chem Chem Phys*. 2017;20(1):206-213. doi:10.1039/c7cp06471c

39. Hansen BB, Spittle S, Chen B, et al. Deep Eutectic Solvents: A Review of Fundamentals and Applications. *Chem Rev*. 2021;121(3):1232-1285. doi:10.1021/acs.chemrev.0c00385

40. Martínez GM, Townley GG, Martínez-Espinosa RM. Controversy on the toxic nature of deep eutectic solvents and their potential contribution to environmental pollution. *Heliyon*. 2022;8(12):e12567. doi:10.1016/j.heliyon.2022.e12567

41. Długosz O. Natural Deep Eutectic Solvents in the Synthesis of Inorganic Nanoparticles. *Materials (Basel)*. 2023;16(2). doi:10.3390/ma16020627

42. Marchel M, Cieśliński H, Boczkaj G. Thermal Instability of Choline Chloride-Based Deep Eutectic Solvents and Its Influence on Their Toxicity─Important Limitations of DESs as Sustainable Materials. *Ind Eng Chem Res*. 2022;61(30):11288-11300. doi:10.1021/acs.iecr.2c01898

43. Abranches DO, Martins MAR, Silva LP, Schaeffer N, Pinho SP, Coutinho JAP. Phenolic hydrogen bond donors in the formation of non-ionic deep eutectic solvents: The quest for type v des. *Chem Commun*. 2019;55(69):10253-10256. doi:10.1039/c9cc04846d

44. Kaoui S, Chebli B, Zaidouni safa, Basaid K, Mir Y. Deep eutectic solvents as sustainable extraction media for plants and food samples: A review. *Sustain Chem Pharm*. 2023;31(November 2022):100937. doi:10.1016/j.scp.2022.100937

45. Çelik S, Kutlu N, Gerçek YC, Bayram S, Pandiselvam R, Bayram NE. Optimization of Ultrasonic Extraction of Nutraceutical and Pharmaceutical Compounds from Bee Pollen with Deep Eutectic Solvents Using Response Surface Methodology. *Foods*. 2022;11(22):1-19. doi:10.3390/foods11223652

46. He T, Zhou T, Wan Y, Tan T. A Simple Strategy Based on Deep Eutectic Solvent for Determination of Aflatoxins in Rice Samples. *Food Anal Methods*. 2020;13(2):542-550. doi:10.1007/s12161-019-01665-7

47. Wu X, Zhang X, Yang Y, Liu Y, Chen X. Development of a deep eutectic solvent-based matrix solid phase dispersion methodology for the determination of aflatoxins in crops. *Food Chem*. 2019;291(March):239-244. doi:10.1016/j.foodchem.2019.04.030

48. Piemontese L, Perna FM, Logrieco A, Capriati V, Solfrizzo M. Deep eutectic solvents as novel and effective extraction media for quantitative determination of Ochratoxin A in wheat and derived products. *Molecules*. 2017;22(1):1-9. doi:10.3390/molecules22010121

49. Dassoff ES, Li YO. Mechanisms and effects of ultrasound-assisted supercritical CO 2 extraction. *Trends Food Sci Technol*. 2019;86(August 2018):492-501. doi:10.1016/j.tifs.2019.03.001

50. Arumugham T, K R, Hasan SW, Show PL, Rinklebe J, Banat F. Supercritical carbon dioxide extraction of plant phytochemicals for biological and environmental applications – A review. *Chemosphere*. 2021;271. doi:10.1016/j.chemosphere.2020.129525

51. Ehsan MM, Awais M, Lee S, Salehin S, Guan Z, Gurgenci H. Potential prospects of supercritical CO2 power cycles for commercialisation: Applicability, research status, and advancement. *Renew Sustain Energy Rev*. 2023;172(May 2021):113044. doi:10.1016/j.rser.2022.113044

52. Uwineza PA, Waśkiewicz A. Recent advances in supercritical fluid extraction of natural bioactive compounds from natural plant materials. *Molecules*. 2020;25(17). doi:10.3390/molecules25173847

53. Tzima S, Georgiopoulou I, Louli V, Magoulas K. Recent Advances in Supercritical CO2 Extraction of Pigments, Lipids and Bioactive Compounds from Microalgae. *Molecules*. 2023;28(3). doi:10.3390/molecules28031410

54. O'Sullivan A, Ryan KM, Padrela L. Production of biopharmaceutical dried-powders using supercritical CO2 technology. *J Supercrit Fluids*. 2022;187(February):105645. doi:10.1016/j.supflu.2022.105645

55. Bitwell C, Sen IS, Luke C, Kakoma MK. A review of Modern and Conventional Extraction Techniques and their Applications for Extracting Phytochemicals from Plants. *Sci African*. 2023:e01585. doi:10.1016/j.sciaf.2023.e01585

56. Cao X, Peng B, Ma S, et al. Molecular Selectivity in Supercritical CO2 Extraction of a Crude Oil. *Energy and Fuels.* 2017;31(5):4996-5002. doi:10.1021/acs.energyfuels.7b00415

57. Quitério E, Grosso C, Ferraz R, Delerue-Matos C, Soares C. A Critical Comparison of the Advanced Extraction Techniques Applied to Obtain Health-Promoting Compounds from Seaweeds. *Mar Drugs.* 2022;20(11):1-40. doi:10.3390/md20110677

58. Dali I, Aydi A, Stamenic M, et al. Extraction of lyophilized olive mill wastewater using supercritical CO2 processes. *Alexandria Eng J.* 2022;61(1):237-246. doi:10.1016/j.aej.2021.04.088

59. Lee R, Smith BA, Roy HM, Leite GB, Champagne P, Jessop PG. Jo ur of. *Algal Res.* 2023:103002. doi:10.1016/j.algal.2023.103002

60. Bañares C, Chabni A, de Donlebún BP, Reglero G, Torres CF. Chemical characterization of pomegranate and alfalfa seed oils obtained by a two-step sequential extraction procedure of expeller and supercritical CO2 technologies. *J Food Compos Anal.* 2023;115(September 2022). doi:10.1016/j.jfca.2022.105040

61. Zougagh M, Ríos Á. Supercritical fluid extraction of macrocyclic lactone mycotoxins in maize flour samples for rapid amperometric screening and alternative liquid chromatographic method for confirmation. *J Chromatogr A.* 2008;1177(1):50-57. doi:10.1016/j.chroma.2007.11.021

62. Zougagh M, Téllez H, Sánchez A, Chicharro M, Ríos A. Validation of a screening method for rapid control of macrocyclic lactone mycotoxins in maize flour samples. *Anal Bioanal Chem.* 2008;391(2):709-714. doi:10.1007/s00216-008-2047-3

63. Liau BC, Jong TT, Lee MR, Chang CMJ. Supercritical fluid extraction and quantification of aflatoxins in Zizyphi Fructus by liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry. *Rapid Commun Mass Spectrom.* 2007;21(5):667-673. doi:10.1002/rcm.2870

64. Josephs RD, Krska R, Grasserbauer M, Broekaert JAC. Determination of trichothecene mycotoxins in wheat by use of supercritical fluid extraction and high-performance liquid chromatography with diode array detection or gas chromatography with electron capture detection. *J Chromatogr A.* 1998;795(2):297-304. doi:10.1016/S0021-9673(97)00944-8

65. Taylor SL, King JW, Greer JI, Richard JL. Supercritical fluid extraction of aflatoxin M1 from beef liver. *J Food Prot.* 1997;60(6):698-700. doi:10.4315/0362-028X-60.6.698

66. Huopalahti RP, Ebel J, Henion JD. Supercritical fluid extraction of mycotoxins from feeds with analysis by LC/UV and LC/MS. *J Liq Chromatogr Relat Technol.* 1997;20(4):537-551.

doi:10.1080/10826079708010943

67. Holcomb M, Thompson HC, Cooper WM, Hopper ML. SFE extraction of aflatoxins (B1, B2, G1, and G2) from corn and analysis by HPLC. *J Supercrit Fluids.* 1996;9(2):118-121. doi:10.1016/S0896-8446(96)90007-8

68. Wu P, Yen Y, Chiou RY. Extraction of Aflatoxin from Peanut Meal and Kernels by CO 2 -Methanol Using a Highly Pressurized Fluid Extraction Process. 1995;58(7):800-803.

69. Taylor SL, King JW, Richard JL, Greer JI. Analytical-Scale Supercritical Fluid Extraction of Aflatoxin B1 from Field-Inoculated Corn. *J Agric Food Chem.* 1993;41(6):910-913. doi:10.1021/jf00030a014

70. Engelhardt H, Haas P. Possibilities and Limitations of SFE in the Extraction of Aflatoxin B1 from Food Matrices. *J Chromatogr Sci.* 1993;31(1):13-19. doi:10.1093/chromsci/31.1.13

71. Teo CC, Tan SN, Yong JWH, Hew CS, Ong ES. Pressurized hot water extraction (PHWE). *J Chromatogr A.* 2010;1217(16):2484-2494. doi:10.1016/j.chroma.2009.12.050

72. Chemat F, Vian MA, Ravi HK, et al. Review of alternative solvents for green extraction of food and natural products: Panorama, principles, applications and prospects. *Molecules.* 2019;24(16). doi:10.3390/molecules24163007

73. Carr AG, Mammucari R, Foster NR. A review of subcritical water as a solvent and its utilisation for the processing of hydrophobic organic compounds. *Chem Eng J.* 2011;172(1):1-17. doi:10.1016/j.cej.2011.06.007

74. Lachos-Perez D, Baseggio AM, Mayanga-Torres PC, et al. Subcritical water extraction of flavanones from defatted orange peel. *J Supercrit Fluids.* 2018;138(March):7-16. doi:10.1016/j.supflu.2018.03.015

75. Kilpeläinen P, Liski E, Saranpää P. Optimising and scaling up hot water extraction of tannins from Norway spruce and Scots pine bark. *Ind Crops Prod.* 2023;192(August 2022). doi:10.1016/j.indcrop.2022.116089

76. Ong ES, Oh CLY, Tan JCW, Foo SY, Leo CH. Pressurized hot water extraction of okra seeds reveals antioxidant, antidiabetic and vasoprotective activities. *Plants.* 2021;10(8). doi:10.3390/plants10081645

77. Krstić M, Teslić N, Bošković P, et al. Isolation of Garlic Bioactives by Pressurized Liquid and Subcritical Water Extraction. *Molecules.* 2023;28(1). doi:10.3390/molecules28010369

78. Gbashi S, Njobeh PB, De Saeger S, De Boevre M, Madala NE. Development, chemometric-assisted optimization and in-house validation of a modified pressurized hot water extraction methodology for multi-mycotoxins in maize. *Food Chem.*

2020;307(May 2019):125526. doi:10.1016/j.foodchem.2019.125526

79. Gbashi S, Njobeh PB, Madala NE, De Boevre M, Kagot V, De Saeger S. Parallel validation of a green-solvent extraction method and quantitative estimation of multi-mycotoxins in staple cereals using LC-MS/MS. *Sci Rep*. 2020;10(1). doi:10.1038/s41598-020-66787-z

80. Wang N, Duan C, Li S, Geng X, Ding K, Guan Y. Aqueous extraction followed by dispersive solid phase extraction with in situ derivatization for the determination of aflatoxins in traditional Chinese medicines. *J Chromatogr A*. 2020;1618. doi:10.1016/j.chroma.2020.460894