Chapter 11

Subcritical Water Extraction and Its Prospects for Aflatoxins Extraction in Biological Materials

Sefater Gbashi, Ntakadzeni Edwin Madala, Oluwafemi Ayodeji Adebo, Lizelle Piater, Judith Zanele Phoku and Patrick Berka Njobeh

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Abstract

Aflatoxins (AFs) are well-known mycotoxins and contaminants of various agricultural commodities globally that are linked to a wide range of adverse health and economic complications. Because of their incessant proliferation and deleterious consequences, it has become mandatory to routinely monitor the levels of these toxins in agricultural products before they go into the market. Essentially, effective analysis is an important component of AFs control, and extraction is a necessary step for their analysis, irrespective of the protocol adopted. Conventional methods for AF extraction are expensive, the processes involved are tedious and utilize large quantities of organic solvents that are environmentally unfriendly. This has necessitated the quest for alternatives that are 'green', cost-effective and easy to perform. In this regard, subcritical water extraction (SWE) is a viable alternative that has proven to be effective in the extraction of other bioactive compounds. This chapter presents a critical appraisal of the principles and dynamics of SWE, and its current applications as a viable tool in the extraction of AFs from various biological matrices. Although further research needs to be performed to enhance its applicability, the adoption of SWE in the extraction of AFs seems very promising and needs to be properly exploited.

Keywords: subcritical water extraction, aflatoxins, temperature, biological materials

1. Introduction

The proliferated contamination of agricultural commodities by AFs has become a serious global concern because of their severe impact on health and the economy [1, 2]. This group of mycotoxins is food contaminants produced by filamentous toxigenic fungal species [3, 4] principally those members within the *Aspergillus* genera. Relative to their contamination of food and feed commodities, approximately 4.5 billion people in the world are at the risk of been chronically exposed to mycotoxins, in particular, AFs [5, 6]. Several reports have implicated AFs as very poisonous human and environmental pollutants [2, 7–10]. In fact, one of the AFs, aflatoxin B_1 (AFB_1), has been recognized as the most potent naturally occurring carcinogen known to man [2]. As a result of their widespread proliferation and associated deleterious effects, there is a growing concern over their intake via consumption of contaminated food and feed by humans and animals alike. This has led to more stringent guidelines and regulatory limits of these toxins, especially with the globalization of the food supply chain, and consequently, necessitating routine surveillance of the prevalence as well as levels of AFs in food and feed [11, 12]. As such, the need for more sensitive and robust analytical methods for the determination of AFs is eminent [12], particularly one that is carried out following the greener route.

Extraction is an important step in AFs analysis. It is inevitable irrespective of the protocol adopted. Although different methods exist for extracting AFs from food and feed such as solvent extraction, solid-phase extraction, and immuno-affinity column (IAC) extraction, there are anxieties over human and environmental health regarding safety in their applications [13, 14]. Conventional techniques also involve labor-intensive and time-consuming procedures [15], requiring relatively large volumes of organic solvents, which are expensive and hazardous [13, 16]. Bearing in mind these concerns associated with extraction of AFs, the design of a greener route that is efficient, cheap, fast and relatively easy to address these challenges is significant. Subcritical water extraction (SWE) seems promising in this regard. Better results, recoveries, and effectiveness have been reported for SWE as compared to other traditional methods for extracting different bioactive compounds [17–19]. In this chapter, a comprehensive review on the implications of AFs and issues with their analysis is presented. The need and potential applicability of SWE in AF analysis are highlighted. Lastly, herein, we demonstrate the basic principle of SWE, underscoring its advantages and disadvantages, and wrapping up the chapter with a discussion on how SWE can be suitable in extracting AFs from biological matrices for routine analysis.

2. Aflatoxins

2.1. Definition and concept of aflatoxins

Aflatoxins are the most perilous and troublesome group of mycotoxins to humans and animals that are generally produced by toxigenic strains of fungi, notably *Aspergillus flavus*, *A. parasiticus* and *A. niger* [2, 20, 21]. At least 14 different types of AFs are known to exist in nature, however, the major ones of economic and health significance are aflatoxin B_1 (AFB_1),

B_2 (AFB_2), G_1 (AFG_1), G_2 (AFG_2), M_1 (AFM_1) and M_2 (AFM_2). AFM_1 and M_2 are hydroxylated metabolites of AFB_1 and B_2, respectively, bio-transformed by the liver and found in milk, urine, and other body fluids, being less harmful than their precursor toxins [22, 23]. Among the AF group, AFB_1 is considered the most toxic. This one has been established as the most notorious naturally occurring carcinogen [2, 24, 25]. For that reason, it has been classified as a Group 1A human carcinogen [26]. Cereals such as maize are common crops that are contaminated by AFs. Additionally, crops such as oilseeds, including peanuts, different kinds of spices, figs and other dried fruit, are also familiar but most susceptible substrates.

2.2. Physicochemical properties of aflatoxins

Aflatoxins are a group of closely related difuranocoumarin derivatives, with similar structures as they constitute a unique group of naturally occurring heterocyclic compounds that are highly oxygenated [22]. AFs can be broadly classified into two groups based on their chemical structure namely, difurocoumarocyclopentenone series (AFB_1, B_2, M_1, M_2, and other derivatives) and difurocoumarolactone series (AFG_1, G_2, and others) [22]. Their chemistry constitutes highly substituted coumarins containing a fused dihydrofurofuran moiety. The AFBs (*i.e.*, members of the blue fluorescent series) generally feature a fusion of a cyclopentenone ring to the lactone ring of the coumarin moiety, while the AFGs possess a fused lactone ring [27]. AFB_1 and G_1 contain an unsaturated bond on the terminal furan ring at the 8, 9 position. Epoxidation at this position has shown to be essential for their carcinogenicity [28]. The intensity of fluorescence (light) emission differs greatly among the four compounds. This property plays a significant role in their quantification by fluorescence techniques [29]. AFs are also very stable chemical compounds and notoriously difficult to eradicate in food commodities [30, 31]. They are chemically stable during processing and storage, even when heated at quite elevated temperatures such as those achieved during the production of breakfast cereals or baking of bread [31, 32]. This necessitates the avoidance of conditions that favor their production, which is not always feasible in practice [31, 33].

2.3. Exposure and health implications of aflatoxins

The presence of AFs in foods and feeds is problematic as it induces vicious health repercussions in humans and animals when exposed to them. Poisoning from AFs has been reported in different parts of the world, and victims include humans, animals and other non-human primates [34]. Common exposure routes include ingestion of AF contaminated foods and feeds; however, aerosol, parental (placental and breastfeeding) and dermal routes have also been reported [9, 35], but it can be supposed that ingestion is the main source of AF exposure among humans and animals. This group of poisons enters the blood stream and lymphatic system with the liver as targeted organ and damage macrophage systems inhibit protein synthesis and increase sensitivity to opportunistic infections [36]. Exposure to AFs can be chronic or acute, and symptoms and degree of illness depend on the type of AF, concentration, and duration of exposure, as well as species, age, sex, and health status of the exposed individual [37].

Aflatoxicosis refers to poisoning and associated illness resulting from AF exposure [38, 39]. There are numerous cases of aflatoxicosis reported in the literature. In Ibadan, Nigeria, the

death of some children who consumed mould-infested *Kulikuli*, was suggested to be due to aflatoxicosis [36]. An outbreak of hepatitis in 1974 in India that killed 100 people and caused ailment in hundreds of others was as a result of AFs via consumption heavily contaminated maize [40]. Incidence of liver cancer and aggravated cases of over 40% of diseases in developing nations including kwashiorkor, growth stunting, and HIV are directly or indirectly associated with dietary AF exposure [5, 27, 41]. One of the most epic episodes of aflatoxicosis reported in human history occurred in rural Kenya, of which 317 cases of illness and 125 deaths were reported [42]. The cause of this outbreak was deciphered to be consumption of maize products heavily contaminated with AFs (several folds above the Kenyan regulatory limit of 20 μg/kg). An outbreak of canine aflatoxicosis occurred in South Africa in 2011 leaving over 220 dogs dead and several others seriously affected after consuming pet food contaminated with high levels of AFs [43]. It is, however, very problematic that aflatoxicosis often remains unrecognized by health workers for an extended period of time, except when a large number of people are affected [9].

2.4. Economic implications of aflatoxins

2.4.1. Economic losses due to aflatoxins contamination of food and feed

The economic significance of AFs is globally illustrious both in the developed and in the underdeveloped nations. In the United States, losses due to AF contamination of maize are estimated at up to 1.68 billion US dollars annually [44, 45]. Globalization of trade has added to the cost and complexity of the situation. For example, adopting the EU standard limit of 4 μg/kg for AFs in peanuts was estimated to cost about 450 million dollars in annual losses on exports [45, 46]. Although estimates on the economic impact of AFs are scarce in the developing countries, based on the literature reports of high levels of mycotoxins found in agricultural commodities in these countries, it is probable that losses consistently far exceed those reported in the United States [45]. To give an instance, in Southeast Asia, the impact of AFs is calculated to a level of 900 million US dollars, of which 500 million are costs directly related to human health effects [45]. In the coastal and eastern regions of Kenya, 2.3 million bags of maize worth over Ksh 3.2 billion (roughly 30 million US dollars) were declared unfit for human consumption by the Ministry of Public Health and Sanitation in 2010 due to high levels of AF contamination [47]. The change in policy by the European Union (EU) is expected to reduce imports on cereals, dried fruits and oilseeds (mainly nuts) by 64%, costing some nine African countries including Egypt, Nigeria and South Africa about 670 million US dollars in trade per year [48]. These economic impacts add to the complexity of the AF malice in developing countries, as they may be compelled to export their best quality produce and sadly retain the poorer commodities for domestic use [47].

2.4.2. Research and surveillance costs of aflatoxins in food and feed

The economic impact of AFs is felt across the entire food and feed supply chains, that is, "from farm to fork." Costs associated with AF management, that is, from sampling and related research expenses, surveillance, mitigation to litigation are also very significant [45, 49]. A

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study conducted in West Africa estimated annual costs averaging 466 million US dollars from testing, regulatory enforcement, to other quality control measures [50]. In 2000, the USDA's Agricultural Research Service (ARS) instituted a mycotoxin research program worth 17.7 million US dollars primarily geared towards prevention of fungal contamination and toxin production in crops [49]. On average, total value of commercially available test kits for AFs on the market is approximately 10 million US dollars annually, whereas the cost for analysis of AFs alone is placed at 30–50 million US dollars on annual basis [49].

2.5. Regulation of aflatoxins

As elucidated earlier in Section 2.4, AFs constitute a major concern to human health and national economies around the world. Due to the fact that AFs are ubiquitous contaminants and potent carcinogens even at low concentrations, they require stringent regulation to ensure food safety and human health. Different countries have established various limits for AFs in agricultural commodities marketed within their jurisdiction based upon their own perception of risk assessment. At present, over 100 countries have regulations in limiting AFs and other mycotoxins in the food and feed industry [51]. AFs are the most regulated mycotoxins, and 61 countries have regulatory limits of AFB_1 in foodstuffs ranging from 1 to 20 μg/kg, 76 countries have limits up to 35 μg/kg for total AFs in foodstuffs, whereas 21 countries have limits of up to 50 μg/kg for total AFs in animal feeds [52]. In South Africa, regulations exist for total AFs in peanuts intended for further processing (15 μg/kg), in ready-to-eat foodstuffs for humans (10 μg/kg of which AFB_1 is not more than 5 μg/kg), and AFM_1 in diary milk (0.05 μg/L) [53, 54]. The Joint Expert Committee on Food Additives (JECFA) of the Food and Agriculture Organization (FAO) and World Health Organization (WHO) serving as a scientific advisory body to CODEX Alimentarius Commission recommended that levels of AFs in food should be kept As Low As Reasonably Achievable (ALARA) [52]. FAO [52] has published a compendia summarizing worldwide regulations for AFs and other mycotoxins. Many other similar synopses on limits and regulations for AFs are available in literature and could be consulted for additional information [55–57].

2.6. Analysis of aflatoxins in food and feed

Due to the severe effects that AFs elicit in animal and man, several countries and politico-economic unions have placed high priority on the safety of agricultural commodities marketed and consumed within their jurisdiction [51, 52, 58]. Particularly with the globalization of trade, much efforts have been put into mitigation and control of the prevalence of this toxin group in food and feed [59–62]; however, it is apparent that the complete elimination of AFs from foods is an unattainable objective [37]. This has led to various interventions put in place to manage and minimize risk exposure to them [63, 64]. Adequate risk management has been identified as a critical frontline defense in the overall control of AFs in food and feed supplies [36, 63, 65–67]. Any good food safety management program for naturally occurring toxicants [such as Hazard Analysis and Critical Control Point (HACCP)] assumes a holistic approach, involving various phases such as determination of exposure levels, establishment of analytical capabilities, setting and ensuring compliance with regulatory limits, and establishment of surveillance programs [36, 66].

Such critical approaches provide for routine and detailed analysis of every step throughout the food supply chain (from farm to fork) [36, 63]. This positions analysis at the epicenter of AFs management and risk control, which is a global priority [11, 68]. Bearing in mind that decisions relating to regulatory issues or commercial arbitration need to be based on well-defined methods of analysis [66], it is thus vital to ensure that methods for AF analysis are sensitive, efficient and validated against standard guidelines [69, 70]. In AF analysis, the role played by extraction and sample preparation, in general, cannot be overemphasized. Extraction is an inevitable step in AFs analysis no matter the analytical method employed. It has been estimated that up to 70% and perhaps even more of the effort and time that goes into sample analysis comprises the extraction and sample preparation process [71]. Proper design of the extraction process facilitates rapid, efficient and quality analytical results [71].

2.6.1. Extraction of aflatoxins

Many efforts have been geared towards developing suitable methods to quantitatively extract and detect AFs in agricultural commodities. For any bioanalytical chemists, the goal is to develop methods with improved sensitivity and selectivity, while at the same time maintaining the credibility of the results, as well as reduce cost and time [72].

2.6.1.1. Conventional extraction methods for aflatoxins in food and feed

Different methods have been used for AFs extraction in food and feed. Of these methods, solvent extraction is one of the oldest but still most frequently used method [73]. This method separates analytes based on their relative solubility in two different liquids that are immiscible [74]. One or more solutes contained in a feed solution are transferred to another immiscible solvent, often by rigorously mixing the two immiscible phases, then allowing the two phases to separate [74, 75]. The enriched solvent is called the extract [76]. Common solvents used for solvent extraction include methanol, acetonitrile, chloroform, ethyl acetate, isooctane, ethanol and dichloromethane [4, 73, 77]. The most commonly used solvent extraction approach for AFs is the multi-mycotoxin extraction method of Patterson and Roberts [78]. This method utilizes different organic solvents and reagents such as acetonitrile, isooctane, potassium chloride, dichloromethane and sulfuric acid. It has been widely favored because it selectively extracts several mycotoxins in a single extraction. However, the application of solvent extraction has been greatly limited because it enables the consumption of large quantities of organic solvents, which pose hazards to the environment [16, 79]. Furthermore, solvent extraction often involves long extraction times and laborious procedures with the process extending up to 24 h or more [4, 78]. Moreover, solvents of the required purity tend to be expensive and there are often additional costs with proper disposal of wastes after use [74, 79].

Solid phase extraction (SPE) is another very commonly used extraction method for AFs. It involves the separation of analytes between a liquid mobile phase and a stationary phase contained in a cartridge. Typical materials used at the solid adsorbent phase include ethyl (C2), octyl (C8), octadecyl (C18), cyanopropyl (CN), aminopropyl (NH2), and an ion exchange phase [80]. Non-specific SPE materials are commonly still employed in AF analysis, which is often used for the extraction of more than one mycotoxin [73]. The use of more analyte-specific stationary

phases such as immunoaffinity (IA) materials that contain specific antibodies that bind to the analyte of interest is also gaining much attention [70, 81]. Although SPE techniques are relatively simple, have higher specificity and require little quantities of solvents, they are also very expensive and the antibodies are not available for some mycotoxins and products [80].

2.6.1.2. Other methods for aflatoxin extraction

Aside from the extraction methods discussed above, several other methods have been investigated for the extraction of AFs, some of which include quick, easy, cheap, effective, rugged, and safe (QuEChERS) [82, 83], supercritical fluid extraction (SFE) [84], ultrasonic extraction [85], and many others reviewed in the literature [12, 70, 73, 86, 87]. However, as elucidated previously in Section 2.6.1.1, these techniques are fundamentally limited by the use of large volumes of organic solvents, some of which are well known to be toxic and considered as environmental hazards, issues of low recovery, long and laborious procedures and high costs involved amongst others [72]. Moreover, novel advancements in spectrometric analysis of bioactive compounds (e.g., "omics") are pushing the limits of conventional techniques of extraction [72, 88].

Further to this, the adoption of an extraction method strongly depends on the analytical objectives; hence, for AF analysis, methods are required to meet established benchmark standards of the survey, monitory work, legislation and research [89]. It is in line with this that we propose the adoption of SWE as an alternative to conventional extraction methods for AFs, particularly with respect to improved recovery and selectivity, reduced organic solvent consumption and extraction time, at a lower cost. SWE has been in the spotlight as an efficacious and highly promising alternative to traditional techniques of extraction, whose successful applications in the biochemical, pharmaceutical and chemical engineering fields have been well documented in the literature [14, 90–96]. A brief description of this method of extraction is presented in the proceeding sections of this chapter.

3. Subcritical water extraction

3.1. Concept and principle of subcritical water extraction

The term subcritical water refers to liquid water between the boiling point temperature and critical point temperature of water (100–374°C) (**Figure 1**) [14]. Pressure is applied to keep the water in liquid state. Subcritical water extraction (SWE) is a green, cheap and easy-to-adopt extraction technique that utilizes water within its subcritical region as the extraction solvent [14]. The phenomenon behind the extractability of subcritical water is based on the fact that when the temperature of water is raised and the pressure kept sufficient to maintain it in its liquid state (e.g., 250°C and 50 bar), the dielectric constant of water decreases and the hydrogen bond and other intermolecular forces of water weakens, which greatly enhancing its extractability [14, 97].

At atmospheric temperature and pressure (25°C at 1 bar), water has one of the highest dielectric constants amongst non-metallic liquids (ε=80) [98]. However, when the temperature and pressure of water are raised to 250°C and 50 bar, respectively, the dielectric constant falls (ε=27), which is around the range of non-polar solvents such as methanol (ε=33), acetone

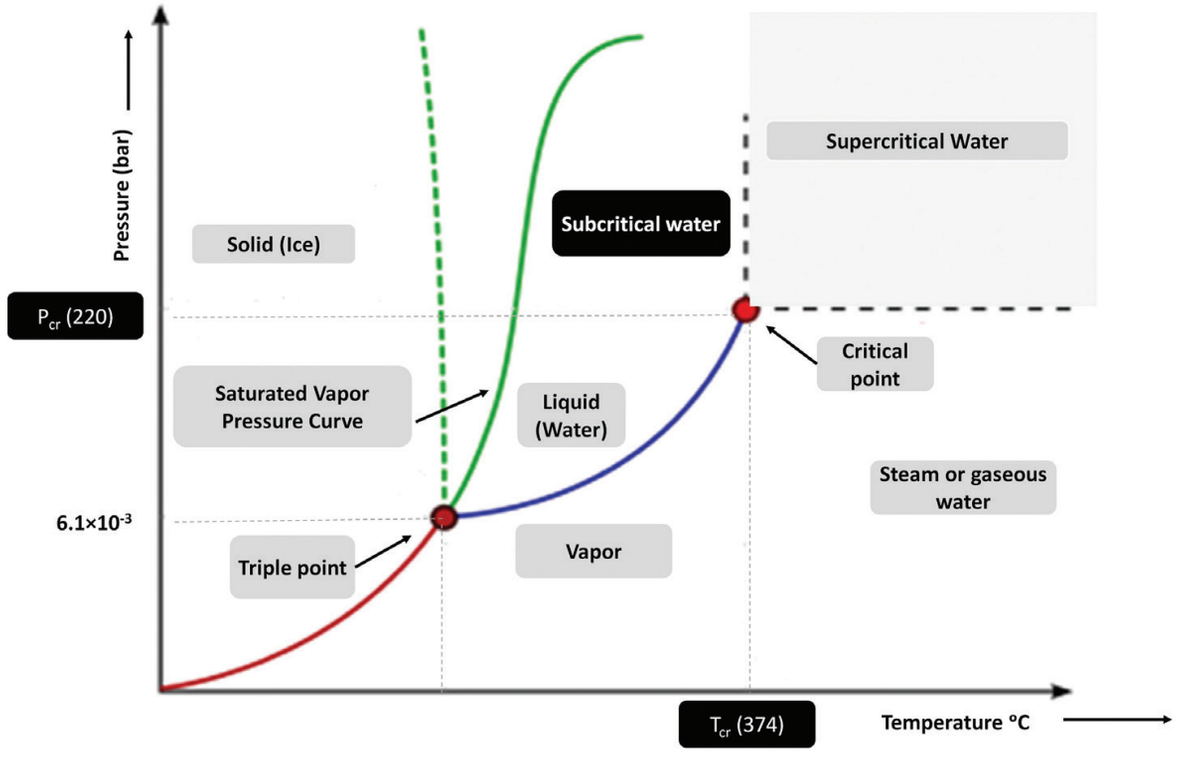

Figure 1. Phase diagram of water as a function of temperature and pressure [14].

(ε=20.7), ethanol (ε=24), and acetonitrile (ε=37) [13, 97]. As a result of the drop in the dielectric constant of subcritical water, its surface tension and viscosity decrease, while its diffusivity increases [13, 14]. As such, water behaves like an organic solvent, dissolving a wide range of low and medium polarity analytes [14]. Interestingly, the extractability and selectivity of subcritical water can be easily maneuvered to extract a range of analytes by simply varying the temperature conditions of the water [90]. Another theoretical explanation on the extractability of subcritical water basis this ability on the fact that, as the temperature of water increases, the average kinetic energy of the molecules of the mixture also increases. This thus disrupts the bonds that exist within and between the molecules, as such, increasing extraction rate.

3.2. Instrumentation of subcritical water extraction

A typical setup of a laboratory scale SWE unit comprises a source of water, temperature retention coil, a solvent pump, an oven and extraction cell, a backpressure valve and a condenser connected to the outlet (**Figure 2**). The grounded sample to be extracted is placed inside the extraction cell, which is located inside the oven. The oven, which usually has an automatic thermostat mechanism, is set to the desired temperature, the backpressure valve is partially locked to maintain the desired pressure and water is pumped at a preset flow rate through the retention coil into the extraction cell. The extraction takes place in the extraction cell as the subcritical water flows through it and mixes with the sample. The hot water extract flows through the condenser and is collected at the outlet [14].

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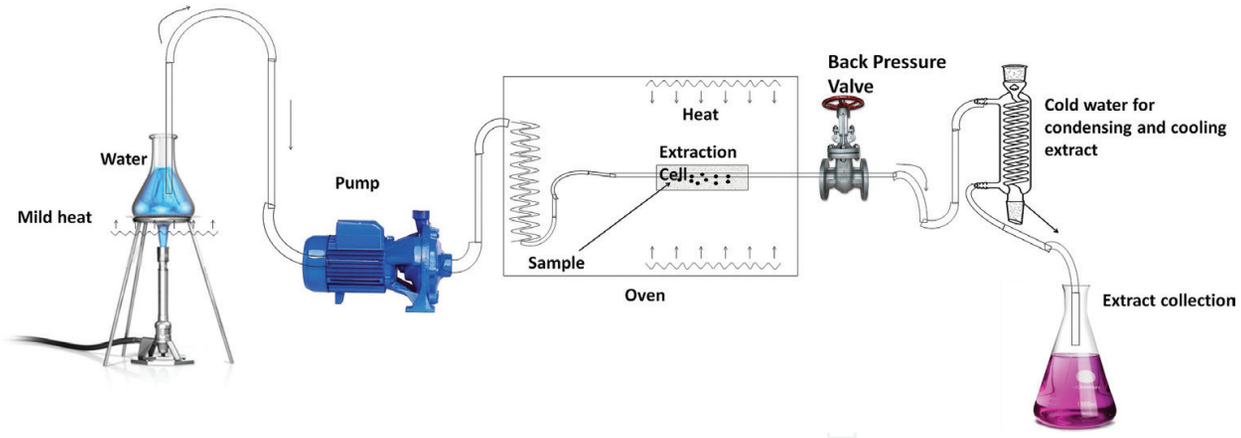

Figure 2. Simple laboratory setup of a PHWE unit [95].

3.3. Operational modes of pressurized hot water extraction

Extraction with subcritical water is performed in two common modes, static mode and flow-through (dynamic) mode. Extraction in the static mode involves retaining the sample in the extraction cell with subcritical water for short periods after which the fluid is allowed to flow out purging the extraction cell and extract collected. It is important to optimize the retention periods to allow for an equilibrium to be reached between the solvent and analyte. The disadvantage of operating in this mode is that within a short time the analyte fluid equilibrium is reached, and hence, no further extraction of the analyte occurs no matter how long the samples are retained in the extraction. On the other hand, extraction in the dynamic mode allows for a continuous flow of fresh fluid through the extraction cell, which reduces or eliminates analyte-fluid equilibrium in a single operation when properly optimized. As such, recovery efficiency is higher in the dynamic mode, although, fluid consumption could be more, resulting in lower energy efficiency compared to the static mode [13, 14]. In a study by Yang *et al*. [99], it was observed that extraction in dynamic mode resulted in the higher recovery of lignin and hemicelluloses from maize stover cellulose than the static mode.

3.4. Factors affecting subcritical water extraction

A number of factors such as temperature, flow rate, pressure, particle size, co-solvents and surfactants affect the performance of SWE. Some of these factors are further described below.

3.4.1. Temperature

The extraction efficiency of SWE is strongly affected by changes in temperature [100]. Generally, extraction efficiency increases with increase in temperature. A higher recovery of total antioxidants was achieved from grape pomace by increasing the extraction temperature [101]. Despite the increase in efficiency by increase in temperature, excess temperatures can result in degradation of thermolabile analytes, hence the need for optimization [100, 102]. The

recovery of carvacrol and thymol from *Zataria multiflora* between 100 and 175°C indicated that recoveries increased steadily with the increase in temperature up to 150°C, then a degradation phenomenon followed with a noticeable burning smell [103].

3.4.2. Pressure

The effect of pressure on the extraction efficiency SWE has been described as insignificant [104, 105]. In a study by Shalmashi *et al*. [106], changes in pressure, that is, 20, 30 and 40 bar during SWE did not show any significant effect on the recovery caffeine from tea waste. This is because water is fairly incompressible at temperatures below 300°C, which implies that pressure has very little influence on the physicochemical properties of water, as long as it can maintain in a liquid state [107, 108]. Nevertheless, increased pressure can compromise matrix tissue membranes and force the extraction fluid deep into matrix pores where water at lower pressure may not normally reach [109].

3.4.3. Cosolvents and modifiers

Cosolvents and solvent modifiers are often used to enhance the extractability of SWE. Cosolvents are secondary solvents (usually organic solvents) that are added to subcritical water to enhance its solvation power [95, 110]. The incorporation of methanol during SWE was observed to significantly ($p < 0.05$) increase yield of flavonoids and di-acylated cinnamic acids from *Bidens pilosa* [95, 111]. Solvent modifiers such as salts and other reagents can alter important physicochemical properties of water such as polarity, surface tension, and hydrogen bonding strength, which results in an enhanced extractability [13, 112]. Modifiers can also interact directly with the sample matrix, reducing the activation energy required for analyte desorption and diffusion [14, 113]. Elsewhere [112], it was observed that the solubility of atrazine can be doubled when urea was added to subcritical water, and when ethanol was used, the solubility increased by over 10-folds.

In addition to the above-described factors, other factors that influence the extractability of SWE include solvent flow rate, physicochemical and functional characteristics of the sample matrix and analyte, matrix particle size and geometry of extraction cell [13, 14, 114].

3.5. Advantages and disadvantages of subcritical water extraction

3.5.1. Advantages of subcritical water extraction

The major advantage of SWE is that it is a green (i.e., environmentally friendly) extraction method. The extractant is water, which is non-toxic, non-flammable and renewable. Moreover, water is readily available and cheap, and extraction with it does not generate harmful by-products [90, 115]. In comparison with traditional extraction methods, SWE is less time-consuming and much easier to perform with very few extraction steps, as such, human errors are greatly minimized. When put side-by-side with supercritical fluid extraction (SFE), SWE edges on the basis of being a simple technology, hence, requiring much lower maintenance and engineering cost for equipment [14, 19]. During extraction with subcritical water, the fluid can be maneuvered to selectively extract a range of analytes with different polarities by mere adjusting the temperature of the water, whereas SFE extracts only nonpolar or light-weight

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compounds [90, 112]. Further to this, SWE is very compatible with various analytical instrumentations because water is colorless and may not interfere with sorts of photodetection such as UV detection or flame ionization detection [14, 116].

3.5.2. Disadvantages of subcritical water extraction

A major setback of SWE is the thermal degradation of some thermolabile analytes at elevated temperatures [117, 118]. When the temperature and pressure of water are extremely high (*i.e.*, above 374°C and 221 bar), there is also the risk that water can become very reactive and could oxidize or catalyze the hydrolysis of some compounds [13]. However, optimization by means of the adoption of a cosolvent or modifier could ameliorate or eliminate these issues [95].

3.6. Application of subcritical water extraction

In the last decade, SWE has been widely investigated for the extraction of various nutritional constituents, organic pollutants, and pharmacoactive compounds from vegetal tissues, food products, soil residues and other ecological biomasses [13, 14, 100, 119]. Free fatty acids and other oils were extracted from spent bleaching earth using SWE [120]. Likewise, it was possible to recover important metabolites from *Moringa oleifera* leaves using SWE [117]. The similar extraction method was used for the recovery of proteins, carbohydrates, and lignans from flaxseed meal [121], catechins and proanthocyanidins from grape seeds [122], flavonoids from aspen knotwood [123] and antioxidants from microalga *Spirulina platensis* [124]. The use of SWE in various applications in different scientific disciplines has been reviewed [90, 100, 107].

3.7. Prospects of subcritical water extraction of aflatoxins

In a recent study [125], we developed and validated an SWE method for the extraction of AFB_1 from maize and subsequently, analysis on high-performance liquid chromatography followed. Results obtained from that study revealed that SWE is suitable for the effective extraction of AFB_1 from maize matrix, with recovery rates ranging from 37 to 128%. Subsequent validation of the optimized method showed acceptable values for accuracy or recovery rate (116%), linearity (%RSD 0.93) and repeatability (%RSD 1.63). It has been stated earlier in Section 2.5 that more countries are enforcing stringent regulations limiting AFs in food and feed, which is increasing demand for their analysis. The efficiency, simplicity, safety and low-cost implications of using SWE are very attractive and compelling in this regard. In comparison with conventional solvent extraction techniques, SWE is very easy to use and requires less time and money [18].

It is known that AFs occur in a diverse manner and can be found deeply deposited inside the food matrices, and as such, their extraction usually requires a process that allows the solvent to penetrate all areas of the matrix to reach hidden toxins trapped in matrix pores [126]. The high pressures involved in SWE seem very suitable in meeting this requirement. Although issues with thermal degradation of some analytes have been a major limiting factor of SWE, it is interesting to know that AFs and most other mycotoxins are relatively thermally stable [31, 62]. Moreover, optimization using cosolvents has been found effective in ameliorating this setback [95, 127]. Accordingly, in our recent study [125] described in the beginning of this section (Section 3.7), we observed a clear positive enhancement on the recovery of AFB_1 by means of a

cosolvent (methanol). Based on these observations and other consulted literature reports, it is evident that SWE is a viable alternative to conventional extraction methods for AFs [14, 125].

4. Conclusion

From the literature documents reviewed herein, it has been established that AFs are very potent natural toxins that constitute a significant nuisance to human and animal health as well as the economy. One way to amply combat the prevalence of these toxic substances is by frequent monitoring of their occurrence levels at various critical points along the food supply chain. To this effect, various national and international regulations have been established and are being enforced. Efficient analytical capabilities provide adequate insights on the prevalence of these toxins, which constitute a basis to monitor and where necessary readdress such interventions. This has positioned analysis as a critical element in AF management and control. Extraction is an important step during AF analysis, and hence, improvement in extraction has been a priority in aflatoxicology research. There is a continual quest for efficient extraction methods that are fast, safe and deliver suitable results at reduced cost. SWE meets all these requirements and could make for efficient routine analysis of AFs and other important fungal metabolites in foods and feeds. These observations could stimulate interest and further propel the adoption of SWE in many other applications even beyond the mycotoxicology domain, as well as its scale-up for subsequent industrial applications.

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Author details

Sefater Gbashi[1]*, Ntakadzeni Edwin Madala[2], Oluwafemi Ayodeji Adebo[1], Lizelle Piater[2], Judith Zanele Phoku[3] and Patrick Berka Njobeh[1]

*Address all correspondence to: sefatergbashi@gmail.com

1 Department of Biotechnology and Food Technology, Faculty of Science, University of Johannesburg, South Africa

2 Department of Biochemistry, Faculty of Science, University of Johannesburg, South Africa

3 Water and Health Research Centre, Faculty of Health Sciences, University of Johannesburg, South Africa

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