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Aflatoxin Occurrence, Contamination, Detection, and Decontamination with Special Emphasis on Coconut Oil: A Review

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ABSTRACT

Purpose: *Coconut oil is one of the commonest and profusely used plant oils in Asian cuisine. Many studies are being carried out aiming at preventing/eliminating potential aflatoxin contamination of the oil or its products along the value chain. The present review analytically provides an overview of aflatoxin occurrence, contamination, detection, and decontamination of vegetable oils with special emphasis on coconut oil.*

Research Method: *Findings and conclusions of studies related to aflatoxins, that are published in authentic sources were reviewed and presented in a chronological manner. Based on the information, current detection and decontamination methods for aflatoxins in edible plant oils were demonstrated.*

Findings: *Complete decontamination of aflatoxins from edible oils seemed impossible, but reducing the accumulated concentrations below the permissible levels seemed possible. The use of chemical agents like alkalis, the most commonly practiced method on a commercial scale, adversely affects human health and the environment. UV irradiation is a promising physical decontamination method of oil with aflatoxins and combining UV irradiation with other potential methods such as the use of adsorbents showed an enhanced efficacy. However, further studies are required to ensure the effective and safe use of biological methods in aflatoxin-contaminated edible oils.*

Research Limitations: *Research gaps in the application of biological decontamination methods in edible oils especially in coconut oil were found.*

Originality/ Value: *This paper critically and aimfully analyzes the relevant information with a view to find gaps thereby showing new directions for applied research to assess nutritional, sensory, and quality attributes in the oils and value-added products, subjected to biological treatments.*

Keywords: *Aflatoxins, Coconut oil, Contamination, Decontamination, Edible plant oils*

INTRODUCTION

Contamination of agricultural commodities by different molds is a serious public health concern worldwide (Karunarathna *et al.,* 2019; Kumar *et al.,* 2021). These molds can produce substances known as mycotoxins which cause adverse effects on the health of humans and animals. Mycotoxins are defined as low molecular weight secondary metabolites synthesized by toxigenic mold species which impart toxic effects to vertebrates in low concentrations (Schaechter, 2009). In most cases, mycotoxins are produced by only a few species within certain fungal genera. Those genera include *Aspergillus*, *Fusarium*,

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Penicillium, *Alternaria,* and *Claviceps* and often contaminate many crops (Karlovsky *et al.,* 2016). According to the data from FAO, the percentage of cereals contaminated due to mycotoxins globally can be approximately 25% (Marin *et al.,* 2013). Among the mycotoxins responsible for food and feed contamination, aflatoxins are the most potent mycotoxin type found in different food commodities (Kumar *et al.,* 2017).

Aflatoxins are extremely potent, naturally occurring carcinogenic secondary metabolites synthesized by different toxigenic fungal strains like *A. prasiticus, A. flavus*, and *A. nomius.* Although 20 different types of aflatoxins have been identified so far, aflatoxins G_1 (AFG_1), aflatoxins G_2 (AFG_2), aflatoxins B_1 (AFB_1), aflatoxins B_2 (AFB_2), and aflatoxins M_1 (AFM_1) often contaminate food commodities affecting human and animal health badly (Tajkarimi *et al.,* 2011; Filazi and Sireli, 2013; Kemboi *et al.,* 2020).

Crops that are often contaminated with aflatoxins include cereals (such as rice, wheat maize, sorghum), oilseeds (such as cotton, peanut, sunflower, soybean, and sesame), nuts (almond, coconuts, Brazil nuts, walnuts, pistachio, fig), spices (chili peppers, black pepper, coriander, turmeric, ginger) and others such as cocoa (Filazi and Sireli, 2013; Fernando *et al.,* 2021). Aflatoxin can also be found in the milk, eggs, and meat of animals fed contaminated feed (Wexler, 2014; Mahato *et al.,* 2019). Aflatoxin contaminations have been reported in food and feed commodities such as maize, wheat, groundnuts, millet, sesame seeds, rice, fig, and some spices owing to fungal infection associated with pre-and post-harvest conditions (Tajkarimi *et al.,* 2011; Guchi, 2015; Mahato *et al.,* 2019).

The negative health consequences of the consumption of food contaminated with aflatoxins include hepatotoxicity, carcinogenicity, teratogenicity, and mutagenicity (Eaton and Groopman, 2013). High consumption rates of oil seed and oil seed-containing products globally have created enhanced consumer attention to high levels of aflatoxins in them. Aflatoxins (particularly, AFG_1, AFG_2, AFB_1, and AFB_2) are capable of contaminating different sources of raw materials from which edible plant oils are produced (Beheshti and Asadi, 2013; Mohammed *et al.,* 2018). Contamination can arise due to improper storage conditions during pre-harvest and post-harvest processing (Tajkarimi *et al.,* 2011). Apart from that, high temperatures and high relative humidity, prevailing consistently associated with tropical countries were found to trigger the growth and development of aflatoxigenic fungal species. This could result in contamination of final edible plant oil products with aflatoxins (Bordin *et al.,* 2014).

Edible plant oils like maize oil, olive oil, peanut oil, sunflower oil, and coconut oil are highly abundant and popular globally, as they are an important dietary source of everyday nutrition. Inappropriate practices in different stages of edible plant oil production such as packaging, transportation, and storage, could encourage mold growth resulting in mycotoxin contaminations in oils (Waqas *et al.,* 2021). In addition, owing to the novel industrial processes, agricultural practices, climatic changes, and environmental pollution, new toxic residues in oil have been increasing (Farré and Barceló*,* 2013). Studies revealed the presence of heavy metals, pesticide residues, and mycotoxins found in culinary oils and fats (Kivevele and Huan, 2015; Tuzimski and Rejczak, 2016; Bhat and Reddy, 2017; Waqas *et al.,* 2021).

Among the edible plant oil types, coconut oil is among the commonest and most abundantly used cooking oil types in many countries including the Philippines, India, Indonesia, Viet Nam, China and Sri Lanka (Krishna *et al.,* 2010). Many studies have evaluated and reviewed aflatoxin contamination in edible plant oils (Banu and Muthumary, 2010; Mariod and Idris, 2015; Mohammed *et al.,* 2018) with hazardous levels of AFB_1 frequently. Therefore, modern science focuses on exploring and investigating various environmentally friendly techniques to detect aflatoxin levels in food products, and mitigating techniques to control the levels of aflatoxins in them. In this context, studies of detection methods

and potential techniques used in preventing the formation of aflatoxins in different types of edible oils are of importance in sustaining the coconut oil industry.

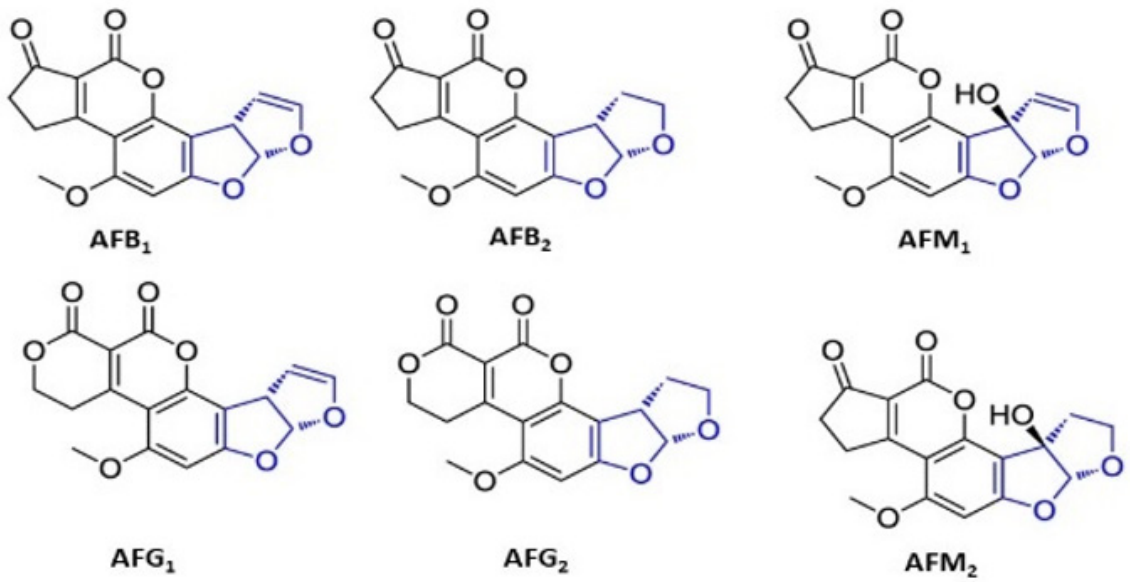
Microbial Sources and Major Aflatoxin Types They Produce

Several *Aspergillus* species including Aspergillus flavus and *A. parasiticus* species produce AFB_1 and AFB_2 (Frisvad *et al.,* 2004; Kumar *et al.,* 2017). The major aflatoxins are produced by *A. flavus, A. nomius,* and *A. parasiticus* (International Agency for Research on Cancer, 2012; Kumar *et al.,* 2017). Depending on the species, the type of toxin they produce may vary. There are more than 20 different varieties of aflatoxins discovered where, AFG_1, AFG_2, AFB_1, AFB_2, AFM_1, and AFM_2 (Figure 01), being the commonest of all (Christensen and Beuchat, 1987; Inan *et al.,* 2007; Kumar *et al.,* 2017).

Studies have revealed that AFB_1, AFB_2, AFG_1, and AFG_2 as the most prominent naturally occurring toxins while AFM_1 and AFM_2 as the hydroxylated metabolites of AFB_1 and AFB_2 (Pitt, 2000; Inan *et al.,* 2007; Hussain *et al*., 2008; Kumar *et al.,* 2017). AFM_1 and AFM_2 can be derived from aflatoxin B types via various metabolic pathways and expressed in animal products (Weidenborner, 2001; Wolf-Hall, 2009; Kumar *et al.,* 2017). Due to the differences between aflatoxins in their structure, the solubility and stability of the toxins may vary.

Among them, aflatoxins AFB_1 and AFG_1, occur more often, while AFB_1, is the most potent. The level of toxicity linked with aflatoxin varies depending on the types present and the order of toxicity is ranked as $AFB_1 > AFG_1 > AFB_2 > AFG_2$ (Jaimez *et al*., 2000; Bandyopadhyay *et al.,* 2007; Kumar *et al.,* 2017).

Generally, each species of *Aspergillus* does not biosynthesize aflatoxin, and based on their genotype they become aflatoxigenic (Smith and Moss, 1985; Pitt and Hocking, 2009). Apart from the biological conditions of *Aspergillus* species, their physical and chemical status affect the production of toxins. Among the aflatoxins identified, AFB_1 and AFB_2 are produced by *Aspergillus flavus* (Table 01), while AFG_1, AFG_2, AFB_1, and AFB_2 are produced by *Aspergillus parasiticus* (Bennett and Klich, 2003; Bennett *et al.,* 2007; Kumar *et al.,* 2017). AFB_1 is highly carcinogenic and active over a wide range of temperatures, including commercial processing conditions (Sirot *et al.,* 2013; Kumar *et al.,* 2017).



Sources: Tajkarimi et al., 2011; Filazi and Sireli, 2013

Figure 01: Structures of the common types of aflatoxins produced by *Aspergillus* species.

Table 01: Specific types of aflatoxins produced by different strains of *Aspergillus.*

Microorganism	Type of Aflatoxin	Toxic effects	References
Aspergillus flavus	AFB_1, AFB_2	Acute liver damage, cirrhosis, carcinogenic, immunosuppressive	Pitt and Hocking, 2009; Kumar *et al.,* 2017.
Aspergillus parasiticus	AFB_1, AFB_2, AFG_1, AFG_2, AFM_1	Acute liver damage, cirrhosis, carcinogenic, G_1 toxicity less than B_1 but greater than B_2	Bennett and Klich, 2003; Bennett *et al.,* 2007; Pitt and Hocking, 2009; Garon *et al.,* 2010
Aspergillus nomius	AFB_1 and AFG_1	Acute liver damage, cirrhosis, carcinogenic, immunosuppressive	Ehrlich *et al.,* 2007; Olsen *et al.,* 2008; Pitt and Hocking, 2009

Abbreviations: AFB_1- aflatoxins B_1, AFB_2- aflatoxins B_2, AFG_1- aflatoxins G_1, AFG_2- aflatoxins G_2, AFM_1- aflatoxins M_1 and AFM_2- aflatoxins M_2

Table 02: Some physiochemical properties of 4 main types of aflatoxin present in edible oils.

Type of aflatoxin	Molecular formula	Melting point (°C)	Molecular weight(g/mol)
AFB_1	$C_{17}H_{12}O_6$	268–269	312.063
AFB_2	$C_{17}H_{14}O_6$	287–289	314.079
AFG_1	$C_{17}H_{12}O_7$	247–250	328.058
AFG_2	$C_{17}H_{14}O_7$	230	330.074

Abbreviations: AFB_1- aflatoxins B_1, AFB_2- aflatoxins B_2, AFG_1- aflatoxins G_1, AFG_2- aflatoxins G_2
Sources: Bennett et al., 2007; Kumar, 2018

According to the most recent classification of aflatoxigenic fungi based on a polyphasic approach, 18 species out of 33 species of *Aspergillus* section *Flavi* are natural producers of aflatoxins. Among those 18 species, 16 species were found to be capable of producing the four major aflatoxin types namely AFB_1, AFB_2, AFG_1, and AFG_2, while the remaining two species are synthesized either from AFB_1 alone or both AFB_1 and AFB_2. The majority of oil seeds such as cotton seed, rape seed, sunflower seed, and coconut are most frequently contaminated with AFB_1, AFB_2, AFG_1, and AFG_2 (Frisvad *et al.,* 2019). The key physiochemical properties of the 4 major aflatoxin types found in edible oils remarkably vary from each other and are illustrated in Table 02.

Influential Factors for Aflatoxin Production in Edible Oils

Contamination of edible oils with aflatoxins has become a serious concern due to their negative effects on human health (Javanmardi *et al.,* 2020) and among many edible oils coconut oil was found to be the mostly affected commodity (Arseculeratne, and de Silva, 1971; Trucksess and Scott, 2008). Studies further revealed that AFB_1, AFB_2 and AFG_1 are frequently found in contaminated coconut oil while AFG_2 was not detected recurrently. Unhygienic practices followed during copra production (raw material) can stimulate the growth of toxigenic fungi on the raw material. In addition, some chemical, biological and environmental factors can

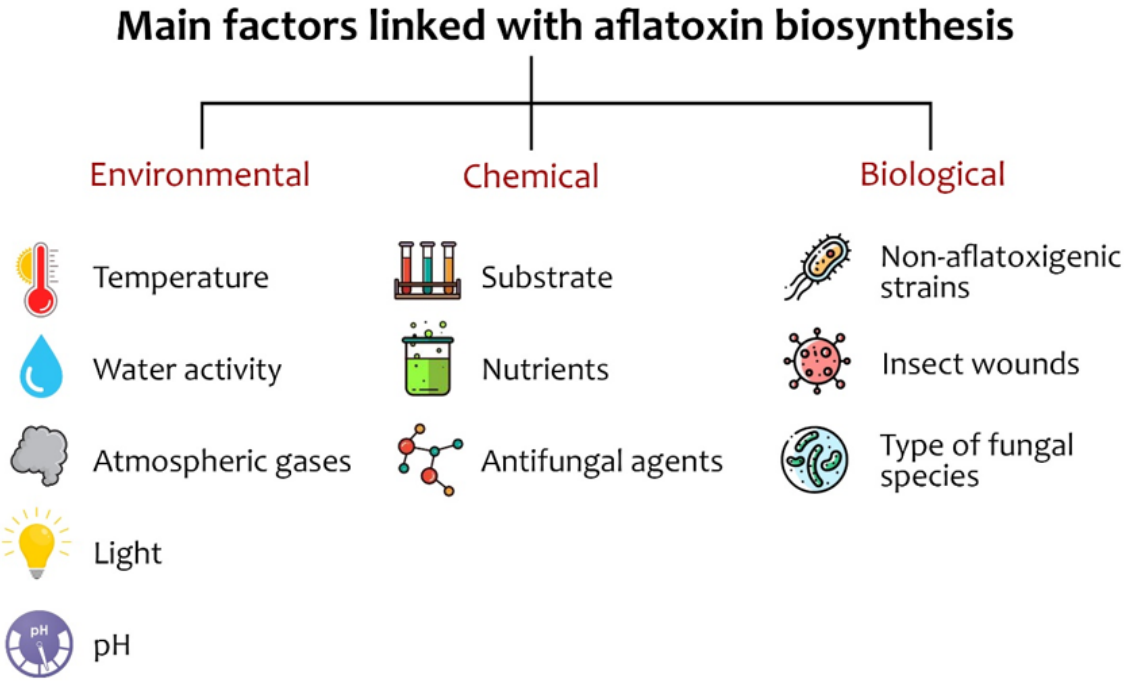
influence aflatoxin biosynthesis and they are depicted in Figure 02. Apart from that, both harvesting (maturity, moisture, and temperature conditions) and storage conditions can have significant impact on aflatoxin occurrence in edible oils (Bryden, 2012; Bhat and Reddy, 2017; Uthpala *et al*., 2020; Fernando *et al.,* 2021).

Biological Factors

Various biological factors can influence the growth of afltoxigenic molds in several ways. Among them, the presence of non-aflatoxigenic competitive strains and damages caused by insects, and the type of fungal species are of significance. In general, insect damage occurring in the crops can cause plant stress and provides suitable ambient conditions for aflatoxin-producing fungi to grow (Kinyungu 2019). According to Huang and others (2011), non-aflatoxigenic strains were found to inhibit the biosynthesis of intraspecific aflatoxin through several mechanisms such as breakdown of already synthesized aflatoxins, inhibition of aflatoxin biosynthesis, competition exclusion, and thigmo response.

Inhibition by non-aflatoxigenic strains: Different species of microorganisms were found to negatively impact the growth as well as aflatoxin synthesis by aflatoxigenic *Aspergillus flavus*. Among them, non-aflatoxigenic strains were regarded as the most potent organisms in the reduction of aflatoxin synthesis (Verheecke *et al.,* 2016; Adebo *et al.,* 2017). For example, it has been found that *A. parasiticus* grows well in the presence of *A. candidus*, and is inhibited in the presence of *A. chevalieri* (Pitt and Hocking, 2009).

Breakdown of synthesized aflatoxins: Recent research supports the fact that several different species of fungi such as *Trichoderma* sp., *Alternaria* sp., *Peniophora* sp., *Phoma* sp., *Armillariella tabescens*, *Mucor* sp., *Rhizopus* sp., *Pleurotus ostreatus*, *Phanerochaete chrysosporium* can result in degrading aflatoxins produced by aflatoxigenic species to less/non-toxic metabolites (Wu *et al.,* 2009; Verheecke *et al.,* 2016; Adebo *et al.,* 2017).



Sources: Arseculeratne and de Silva, 1971; Moreau, 1979; Chavan et al., 2008; Pitt and Hocking, 2009; Yoshinari et al., 2010; Degola et al., 2011; Huang et al., 2011; Bryden, 2012; Bhat and Reddy, 2017; Fernando et al., 2021; Uthpala et al., 2021

Figure 02: Major factors linked with aflatoxin biosynthesis.

Competition with other microflora - competitive exclusion: Recent studies carried out have found that there was a significant decrease in aflatoxin concentration when both aflatoxigenic and non-aflatoxigenic strains were co-inoculated. The results suggested that amount of aflatoxigenic fungi (AF1) was significantly lower (< 50%) when co-inoculated with non-aflatoxigenic strains such as MU_3, B_4, RA_3 and SO_1 (Rao *et al.,* 2020). This confirms that the above-mentioned non-aflatoxigenic strains can interfere with the production of aflatoxin both physically and competitively by excluding the strains responsible for aflatoxin production. The decline of aflatoxin concentration, in the case of co-inoculation can be due to the crowding effect of spores (Degola *et al.,* 2011). Furthermore, studies have proven that the time spent for inoculation of fungi is crucial factor to inhibit aflatoxin synthesis (Degola *et al.,* 2011; Huang *et al.,* 2011).

Thigmo responses: The sensation of touch is essential for fungus in interpreting their surroundings and frequently marks the transition to a different developmental stage (Almeida and Brand, 2017). Studies have found that the contact sensing, or thigmo-based responses of mycelia of different aflatoxigenic and non-aflatoxigenic strains of fungi, was responsible to decrease the production of aflatoxin (Wicklow *et al.,* 2003; Huang *et al.,* 2011).

Environmental Factors

External environmental factors play an important role in the biosynthesis of aflatoxins. The main factors that affect the production of aflatoxins include water activity, temperature, pH, light and atmospheric gaseous composition. *Aspergillus* strains such as *A. flavus* and *A. niger* are found to frequently cause complications (shrinking, discoloration, germination, rotting, and germination) with oilseeds taken for commercial edible oil production leading to toxigenic aflatoxin production in them (Chavan *et al.,* 2008).

In general, aflatoxins production can take place in a wide range of temperatures; nevertheless, the optimal temperatures are in the range of 25-35 °C (Siciliano *et al.,* 2017). It has been reported that, AFB production is high at high temperatures than AFG, however, at low temperatures both AFB and AFG have reported nearly equal production (Matumba *et al.,* 2015).

The optimum conditions for aflatoxin synthesis by *A. flavus* and *A. parasiticus* are 33 °C and 0.99 a_w (Milani, 2013). However, A. *flavus* is capable of producing aflatoxins between 28 and 35 °C (Yu *et al*., 2008), 0.82–0.97 aw (Pitt and Hocking, 2009), and *A. parasiticus* 20–40 °C with aw 0.90 (Schmidt-Heydt *et al.,* 2010).

The pH value of the growth medium is another external factor to affect aflatoxin production (Dalié *et al.,* 2010). During fungal growth in a specific medium, pH may vary to values of 4 to 5 depending on fungal metabolic activities (Moreau, 1979; Uthpala *et al.,* 2021). Researchers have shown that *A. flavus* and *A. parasiticus* species were capable of growing over wide range of pH values ranging from 1.7 to 9.34, with optimum growth occurring in pH values between 3 and 7 (Yoshinari *et al.,* 2010). Although, lower pH values in the range of ($3 > pH > 1$) can retard fungal growth, higher pH values in the range of ($6 > pH > 3$) were shown to promote both fungal growth and aflatoxin synthesis (Eshelli *et al.,* 2015).

Both the fungal growth and the aflatoxin synthesis are impacted by the presence/ absence of light. Darkness was found to enhance aflatoxin production while sunlight inhibits its production. (Rushing and Selim 2019). Apart from that, the availability of atmospheric gases including O_2 and CO_2 is detected to be a significant factor in aflatoxin production. In this regard, both fungal growth and aflatoxin synthesis are inhibited at high levels of CO_2 and low levels of O_2 (Mahbobinejhad *et al.,* 2019).

Chemical Factors

Several chemical factors such as availability of nutrients, type of substrate and presence of antifungal agents are responsible for aflatoxin production by aflatoxigenic fungi. Nutritional factors like carbon, nitrogen, amino acids and some trace elements were found to affect the synthesis of mycotoxin. Simple sugars such as glucose and maltose have been found to contribute to toxin production (Cuero *et al.,* 2003; Yu *et al.,* 2003). Studies revealed that tryptophan inhibited tyrosine formation which assisted in the generation by *A. flavus* (Yu *et al.,* 2007). Production of aflatoxins is directly associated with the number of double bonds in molecules of free and esterified fatty acids. In this regard, linoleic acid and trilinolein are capable of prompting the production of aflatoxins in greater proportion than oleic acid, triolein, and other saturated fatty acids and triacylglycerols. Furthermore, lipid oxidation in the presence of fatty acid hydroperoxides was detected to increase the biosynthesis of aflatoxins (Bircan, 2006).

Liu *et al*., (2016) revealed that the removal of lipids from ground substrates significantly reduces the capability of AFB_1 production by *Aspergillus flavus*. The same work revealed that substates like maltose, glucose, sucrose, arginine, glutamic acid, aspartic acid, and zinc could significantly trigger AFB_1 production. And stachyose more significantly promoted *A. flavus* growth than the other nutrients. Furthermore, antifungal agents like Sorbic acid (Alcano *et al.,* 2016) and propionic acid (Brožková *et al.,* 2015) have shown some inhibitory activity against *A. flavus* and *A. parasiticus*.

Recent Global Aflatoxin Outbreaks in Food and Feed

Hepatocellular carcinoma (HCC) or liver cancer, is the third leading cause of cancer deaths worldwide, with a prevalence 16–32 times higher in developing countries than in developed countries (Liu and Wu, 2010). Aflatoxin, a contaminant produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus* in commodities such as maize and nuts, is a known human liver carcinogen.

Further, it has been found experimentally, that long-term consumption of aflatoxins contaminated food leads to 'aflatoxicosis', which is toxic hepatitis leading to jaundice and in severe cases, death. Even though aflatoxicosis outbreaks can cause severe health issues and death at once, it is also possible to prevent them by careful assessment of vulnerable food sources. As mentioned earlier, the occurrence of liver cancers is found to strongly correlate with diets contaminated with aflatoxins (Williams *et al.,* 2004). Statistics of the global cancer observatory of the International Agency for Research on Cancer (IARC), WHO have revealed that 841,080 new cases of liver cancer were recorded causing 781,631 deaths globally in 2018. African and Asian continents, are among the leading territories, in terms of annual new cases recorded with 64,779 and 609,596 cases respectively, altogether depicting approximately 80% of the total cases reported globally. Literature supports the fact that AFB_1 alone, had resulted in 25,200 to 155,000 cases every year (Liu and Wu, 2010), whereas 40 percent of the cases were recorded in sub-Saharan Africa only (Williams *et al.,* 2004), highlighting that aflatoxin-induced liver cancer is the major cause for one-third of all liver cancers filed in entire Africa (Gibb *et al.,* 2015). Moving down to the country level, China has shown the highest number of liver cancer cases globally owing to two main synergistic reasons; high exposure to dietary aflatoxins and hepatitis B chronic infections (McGlynn and London, 2005).

An acute aflatoxicosis outbreak was reported recently in the central part of Tanzania in the year 2016 (Kamala *et al.,* 2018). The climate in the central region of Tanzania is somewhat hot and semi-arid and often subjected to drought and flood conditions (Anyamba *et al.,* 2019). This climatic condition creates, favourable growth environment for emergence of microbial

pathogens responsible for aflatoxin production. This aflatoxicosis outbreak severely affected on sixty-eight individuals and caused twenty of them to die, unfortunately (Kamala *et al.,* 2018). In the North-eastern region of Tanzania from the period of June to July 2017, two clusters of eight children in Kiteto District, were hospitalized on suspicion of aflatoxicosis (News Desk, 2017). In the year 2013, many newspapers reported aflatoxicosis outbreaks in European countries like Europe, Romania, Serbia and Croatia due to contamination of milk with aflatoxins. In the year 2004, a severe aflatoxicosis outbreak occurred in the East-Central (Makueni-Kitui-Machakos-Thika) region of Kenya. It was violent enough to report three hundred seventeen cases and caused hundred twenty-five deaths. By this outbreak, Makueni and Kitui districts were the most severely affected districts with 47% and 32% of cases, respectively. Next Machakos and Thika districts next highest percentages of 6% and 4% among the total cases.

Aflatoxin Contamination of Coconut Oil in Sri Lanka

Coconut oil (extracted from copra) is the most commonly consumed vegetable oil in Sri Lanka (Mendis *et al.,* 2001; Karunarathna *et al.,* 2019). According to statistics, Central Bank of Sri Lanka (2021), the production of coconut oil in Sri Lanka was around 44,648 metric tons and it is being gradually increased. Hence, contamination of food products with aflatoxins is considered one of the dangerous food safety risks in Sri Lanka (Bandara and Kumari, 2020; Central Bank Sri Lanka, 2021). Coconut-based products, contaminated with aflatoxins were first observed in the Sri Lankan market in the 1960s (Karunarathna *et al.,* 2019). Conceding the industries based on copra production, one of the biggest issues they face is aflatoxin contamination during the drying stage of coconut kernel which leads to copra production (Karunarathna *et al.,* 2019). Improper practices during this post-harvest handling stage are possibly the most scientific explanation behind the aflatoxin prevalence in coconut oil available in the market. Proving this fact, some researchers have reported high levels of AFB_1 contamination in the range of 0.05 mg/kg to 28 mg/kg with a heavy growth of *Aspergillus* spp. in a few copra samples (Arseculeratne and Silva, 1971; Karunarathna *et al.,* 2019).

In the coconut oil manufacturing process, fresh coconut kernel containing a high percentage of moisture is sun-dried and then subject to hot air curing to make copra with a moisture content to the required level (less than 6 %) (Mohanraj and Chandrasekar, 2008). Because of less hygienic practices applied during the drying process of coconut to get copra and storage, *Aspergillus* fungi can grow and produce aflatoxins (Samarajeewa *et al.,* 1983). Moreover, incomplete sun drying of coconut kernels, drying under rainy climate, unhygienic handling practices, pest attacks due to improper storage, storage in damp/wet rooms, and poorly ventilated rooms may encourage fungal growth in copra causing a higher frequency of aflatoxin occurrence (Arsecularatne *et al.,* 1976; Samarajeewa *et al.,* 1983 Karunarathna *et al.,* 2019). Therefore, the aflatoxins produced then have the possibility of getting transferred to the final product in the oil extraction process (Samarajeewa *et al.,* 1983; Marina *et al.,* 2009; Karunarathna *et al.,* 2019). Controversially, virgin coconut oil is extracted directly from coconut milk of the fresh matured coconut kernel without using dried kernels (Marina *et al.,* 2009), thereby, limiting the chances of fungal growth and subsequent aflatoxin development.

Previous studies have reported aflatoxin contamination of coconut oil found in the Sri Lankan market (Table 03).

Table 03: Incidences of contamination of Sri Lankan coconut oil samples with aflatoxins.

Contamination reported year	Amount of Aflatoxin	Recommended permissible maximum limit (Rpml)	Percentage of detected samples/ number of samples	Tested method	References
1971	AFB_1 level of 50 - over 1000 µg/kg	AFB_1 30 µg/kg according to FAO/ WHO/ UNICEF	60%	Aflatoxin assay- (TLC run followed by visual comparison with standards under 365nm UV light)	Arsecularatne and de Silva, fulfill1971
1975	AFB_1 level of 50 µg/kg (mean value) Range of aflatoxin 0 - 400 µg/kg	AFB_1 30 µg/kg according to FAO/ WHO/ UNICEF	116	Aflatoxin assay	Samarajeewa, 1975
1983	AFB_1 level of 186 µg/kg (mean) AFB_1 in the range of 500 - 5000 µg/kg	AFB_1 30 µg/kg according to FAO/ WHO/ UNICEF	100% 9%	Aflatoxin assay	Samarajeewa *et al*., 1983
1983	AFB_1 50 - 1000 µg/kg	AFB_1 30 µg/kg according to FAO/ WHO/ UNICEF	45%	Aflatoxin assay	Samarajeewa and Arseculeratne, 1983
2019	AFB_1 level over 2 µg/kg	Total aflatoxins (4 µg/kg), AFB_1 (2 µg/kg) EU regulatory limit	34%	ELISA and HPLC-FLD method	Karunarathna *et al.,* 2019

Abbreviations: AFB_1- aflatoxins B_1, FAO- Food and Agriculture Organization, WHO-World Health Organization, EU- European Union, UNICEF- United Nations Children's Fund, ELISA-Enzyme linked Immunoassay, HPLC-FLD – High Performance Liquid Chromatography- Fluorescence Detector, TLC- Thin Layer Chromatography

Arsecularatne and de Silva (1971) have revealed that the aflatoxin contamination in 60% coconut oil samples tested was in the range of 50 µg/kg to over 1000 µg/kg. Another study has shown a mean Aflatoxin B_1 level of 50 µg/kg (range less than 1 µg/kg to 400 µg/kg) in 116 coconut oil samples (Samarajeewa, 1975), and a mean Aflatoxin B_1 level of 186 µg/kg in 115 coconut oil samples, ten of which had Aflatoxin B_1 contamination in the range of 500 to 5000 µg/kg (Samarajeewa *et al.,* 1983). A study carried out by Samarajeewa and Arseculeratne (1983) reported that 45% out of total coconut oil samples tested, to contain aflatoxin B_1 contamination in the range of 50 to 1000 µg/kg (Table 03).

In recent research carried out in Sri Lanka, 37.5% tested samples were detected for aflatoxin contamination. Out of those, 31% exceeded the EU maximum permissible level for total aflatoxins (4 µg/kg) while 34% samples exceeded the EU maximum permissible level for AFB_1 (2 µg/kg). It was also revealed that the primary method of oil extraction practiced for all those positive coconut oil samples was through copra. Moreover, all 37% of samples were contaminated with AFB_1, AFB_2, and AFG_1, where AFB_1 was the most significant and AFG_2 being undetected in any of those. These results indicate that some imported or locally produced coconut oil samples available in the Sri Lankan market may pose a

greater health risk to consumers due to aflatoxin contamination (Marina *et al.,* 2009; Karunarathna *et al.,* 2019). Nevertheless, in 2021, the Sri Lanka Standards Institution (SLSI) reported that several consignments of coconut oil imported contained high aflatoxin levels exceeding the maximum recommended level (Gunawardena, 2021). Long-term consumption of these types of oils can cause severe health issues. Hence prevention and proper pre- and postharvest practices are of significance in overcoming these health issues.

The introduction of a systematic approach for monitoring aflatoxin into the regulatory scope for copra used in coconut oil production would be a beneficial step in ensuring consumer safety. According to researchers, attempts to eliminate/ reduce aflatoxin contamination in food consumed by people, will also be an attempt to fulfil UN sustainable goals as food safety is an integral part of food security and healthy lives (Karunarathna *et al.,* 2019). Furthermore, those attempts will enhance the demand for Sri Lankan coconut oil production not only in the local market but also in foreign markets causing a surge in foreign revenue generation into the country.

Human Toxicology of Aflatoxins

Aflatoxin contamination that occurs through food and feed consumption to humans and animals, depicts a significant association with certain chronic and acute illnesses resulting from them (Afsah-Hejri *et al.,* 2013). Depending on the type of aflatoxin and type of fungi responsible for its production, both the level of toxicity and the toxicological consequences change drastically. Out of all identified aflatoxin types, AFB_1 is the most toxic one, therefore, many complete reviews are available regarding its toxicological effect (Abrar *et al.,* 2013; Kew, 2013; Magnussen and Parsi, 2013; Marroquín-Cardona *et al.,* 2014; Valencia-Quintana *et al.,* 2014; Battilani *et al.,* 2016; Kowalska *et al.,* 2017; McCullough and Lloyd, 2019; Rushing and Selim, 2019). According to the International Agency for Research on Cancer, AFB_1 is both hepatotoxic and immunosuppressive and can result in impaired productivity and reproductive efficiency (WHO, 2017; El. Khoury *et al.,* 2019) and various neurological disorders like neurodegenerative diseases and neuroblastoma in humans (Hayashi *et al.,* 2018; Alsayyah *et al.,* 2019).

In addition, chronic exposure to aflatoxins was found to be the cause of a range of other serious disease conditions such as teratogenicity, mutagenicity, and cytotoxicity in mammalians (Smith *et al.,* 2017; Klvana and Bren, 2019). Furthermore, AFB_1 is recognized as the most potent naturally occurring liver carcinogen. Even though there is a close association between dietary uptake of aflatoxins and the occurrence of primary liver cancers like HCC and bile duct hyperplasia (McGlynn and London, 2005), other organs (e.g., pancreas, bladder, kidney, etc.) of the human body have also shown susceptibility of developing cancer cells upon exposure to the mycotoxins (Fouad *et al.,* 2019). AFB_1 has also been reported for its capability of transforming to more toxic precursors in food or after ingestion inside the body (Deshpande, 2002).

Apart from that, exposure to aflatoxins has been found to cause occupational cancers (Marchese *et al.,* 2018). Recently, researchers have been able to find a significant association between exposure to AFB1 and childhood stunting, nutritional disorders like kwashiorkor and growth faltering by causing interruptions to the absorption of micronutrients protein synthesis, and metabolic activities, leading to serious health issues (Williams *et al.,* 2004; Turner, 2013; Knipstein *et al.,* 2015).

Aflatoxin M1 (AFM1) is the principal hydroxylated aflatoxin metabolite found in the milk of dairy cows fed with feed contaminated with AFB1, and the metabolite is also found in the breast-milk of mothers who consume toxin-contaminated foods (Neal *et al.,* 1998).

According to the classification of the International Agency for Research on Cancer (IARC), AFB_1 and AFM_1 as human carcinogens belonging to

Group 1 and Group 2B respectively, with the formation of DNA adducts, segments of DNA bound to a cancer-causing chemical (Marchese *et al.,* 2018).

Detection Techniques of Aflatoxin in Edible Plant Oils

The identification of aflatoxin in coconut oil samples is important for the controlling process of food hazards (Kumar *et al.,* 2017). There are various methods have been evaluated to detect aflatoxin content in contaminated food using several techniques. These techniques include chromatographic, immunochemical, and spectroscopic methods. Further these methods can be categorized into chemical analysis, biological identification, instrumental analysis, and immunoassays (Yan *et al.,* 2020). Moreover, there are factors to be considered when selecting the quantifying method of aflatoxins such as specificity, sensitivity, reliability, and simplicity (Mahfuz *et al.,* 2018). Aflatoxins are controlled in multiple countries and the legal limits range from 0 to 50 ng/g (Kolosova *et al.,* 2006; Mahfuz *et al.,* 2018). The countries like ours have a high degree of aflatoxin exposure due to limited access to sophisticated analytical methods. The Association of Official Analytical Chemists (AOAC) and the European Committee for Standardization (ECS) have published nearly 50 different aflatoxin detection methods for food commodities (Yan *et al.,* 2020). Nevertheless, to ensure the safeness of food and minimize aflatoxin-related sicknesses, the detection methods are required to be specific, sensitive, and also affordable. Every aflatoxin detection method has its benefits and barriers (Mahfuz *et al.,* 2018). There are different methods of aflatoxin detection based on different principles (Table 04).

Aflatoxins are generally identified based on the absorption and emission spectra, by peak absorbance at 360 nm. The type B aflatoxins show blue fluorescence at 425 nm, while aflatoxin type G exhibit green fluorescence at 450 nm under UV light. The fluorescence emission of the aflatoxin type G is ten times greater than that of the aflatoxin B type (Alcaide-Molina *et al.,* 2009). The aforementioned fluorescence appearance is widely admitted for the detection process of aflatoxins. Thin-layer chromatography or TLC is the most traditional method applied for aflatoxin detection (Sapsford *et al.,* 2006; Fallah *et al.,* 2011; Kumar *et al.,* 2017; Karunaratnna, 2019). Sophisticated methods such as HPLC (High-performance liquid chromatography), LCMS (Liquid chromatography-mass spectroscopy), and ELISA (Enzyme-linked immune-sorbent assay) are most frequently used to detect and quantify aflatoxins (Tabari *et al.,* 2011; Andrade *et al.,* 2013; Sulyok *et al.,* 2015; Kumar *et al.,* 2017). Among the currently applied methods, immuno-affinity chromatography (IAC) clean-up along with HPLC is the most frequently used technique for the measurement of aflatoxins (Zheng *et al.,* 2016; Kumar *et al.,* 2017).

Detoxification Methods of Aflatoxins in Edible Plant Oils

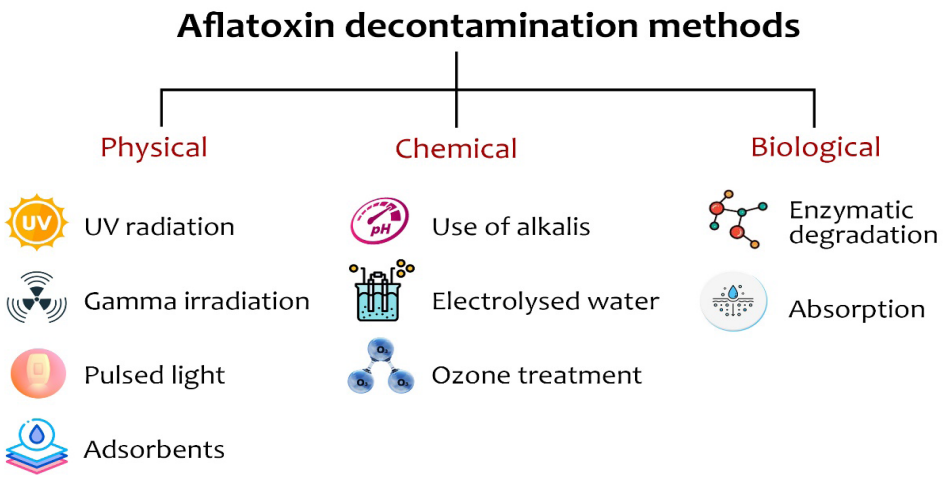
Owing to the increased number of fatal cases reported globally every year due to ingestion of food commodities contaminated with aflatoxins, there is a growing need in the food sector to prevent potential contamination of food products by aflatoxigenic fungi or to inhibit/ control their growth through manipulation of the external environment they live. Apart from that, these control methods can be directed either to decrease the aflatoxin concentration to general safe levels or to synthesize non-toxic/ less toxic degradation products without harming the nutritional composition of treated edible oils. This section will focus on different techniques available to decontaminate aflatoxins in different kinds of edible oils available in the market. The decontamination methods basically fall under three main categories; chemical, physical and biological agents. Most commonly used physical detoxification methods in the edible oil industry include the use of absorbents, gamma irradiation, use of pulsed light, and UV irradiation (Peng *et al.,* 2018). Most recent research work suggests,

the potential of administering chemical agents like alkaline solutions of sodium or potassium hydroxide, ozone, and electrolyzed water to decontaminate aflatoxin-contaminated edible oils. However, so far, none of the studies has been dedicated to investigating the efficacy of biological agents in detoxifying aflatoxin-contaminated edible oils. Meanwhile, none of the studies has been dedicated to estimating the feasibility of applying biological techniques to detoxify aflatoxins present in edible oils (Javanmardi *et al.,* 2020). A brief summary of the common and potential decontamination techniques used in the edible oil industry is shown in Figure 03.

Table 04: Summary details of aflatoxin detection methods.

Method	Benefits	Barriers	References
TLC	Simple equipment, low cost and easy operation	Cumbersome steps, poor sensitivity, need another technique for quantification	Sapsford, *et al.,* 2006; Vosough *et al.,* 2010; Yan *et al.,* 2020; Mahfuz *et al.,* 2018
HPLC	Good repeatability, low detection limit and high sensitivity	Needs derivation, complex operation and high instrument cost	Yan *et al.,* 2020; Mahfuz *et al.,* 2018
LC-MS	Simple pre-treatment, high selectivity and multicomponent analysis	An extremely expensive; very complex machinery	Mahfuz *et al.,* 2018
ELISA	cost effective, limited use of organic solvents, Large number of samples can be analyzed simultaneously, high sensitivity and accuracy	Short reagent life, possibility of false positives/negatives	Yan *et al.,* 2020; Mahfuz *et al.,* 2018
IAC	This method combination with liquid fluorometry is comparable to LC for determination of aflatoxins	Sample destruction, limited to analysis of total aflatoxin	Jaimez *et al.,* 2000; Mahfuz *et al.,* 2018
Biosensors	Rapid, no clean-up procedure, high selectivity and low limit of detection, ease of use, low cost and portability, self-contained, simple design	Cross-reactivity with related mycotoxins, extraction sample prep for solid samples, need measures to improve sensitivity	Sapsford, *et al.,* 2006; Suri *et al.,* 2009; Mahfuz *et al.,* 2018
Quick tests	Swift and simple, do not employ any costly equipment, limited use of organic solvents, suitable for screening purposes	Cross-reactivity with kindred mycotoxins, possibility of false positives/false negatives, poor sensitivity	Cucci *et al.,* 2007; Yan *et al.,* 2020; Mahfuz *et al.,* 2018
LFIA	Fast detection speed, low cost, easy operation, simple equipment	Poor repeatability, difficult to quantify, poor sensitivity	Yan *et al.,* 2020; Mahfuz *et al.,* 2018

Abbreviations: TLC- Thin-layer chromatography, HPLC- High-performance liquid chromatography, LCMS- Liquid chromatography-mass spectroscopy, ELISA- Enzyme-linked immune-sorbent assay, IAC- immuno-affinity chromatography, LFIA- lateral flow immunoassay



Sources: Liu et al., 2011; Abuagela et al., 2018; Peng et al., 2018; Javanmardi et al., 2020; Khaneghah et al., 2021

Figure 03: Aflatoxin detoxification methods used in edible plant oil industry.

Physical Methods

UV irradiation: Fernando *et al*., (2021) have investigated the efficacy of UV irradiation in the detoxification of aflatoxin-contaminated edible plant oils. The degradation of aflatoxins in coconut oil by sunlight was achieved when the coconut oil layer (thickness - 2 mm) was made to expose to sunlight for a period of 10 minutes under laboratory conditions. The results exhibited a relatively higher percentage reduction of aflatoxin levels up to 75% (Javanmardi *et al.,* 2020). Similarly, Diao *et al*., (2015) reported a reduction of aflatoxins up to 45 % when peanut oil was treated with UV light for 2 hours. And also, aflatoxin AFB_1 in peanut oil was found to degrade entirely within ½, an hour under the intensity of 800 mw/ cm^2 (Liu *et al.,* 2011).

Gamma radiation: It is used as one of the effective physical methods of inactivating mycotoxins in edible oils (Khaneghah *et al.,* 2021). Although particularly high doses of gamma radiation (generally greater than 30 KGy) are capable of destroying aflatoxins entirely present in oils, they can also result in deterioration of several parameters such as iodine value (degree of unsaturation), peroxide value (formation of primary oxidation products), acid value (free fatty acids formation because of rancidity) which are key parameters of the shelf-life and, the number of tocopherols and oxidation stability. Therefore, to achieve expected objectives optimally, gamma rays can be used along with other techniques. Recent research reported that Gamma irradiation at doses of 10 kGy could eliminate aflatoxigenic fungi fully, while substantially reducing AFB1 content, without causing a significant difference in tocopherols content, oxidation stability, and overall quality of soybean oil samples tested (Javanmardi *et al.,* 2020).

Pulsed light: Pulsed light (PL) spreads in a wide range of wavelength from 100 to 1100 nm and includes visible (26%), infrared (20%) and UV (54%) regions. The success of this method depends on the synergy effect of photochemical treatments and heat used in the technique. In this regard recent research attempts have revealed the probable application of PL as a decontamination technique for aflatoxins present in peanut oil to attain up to 48.8%, 55.6%, and 78% under rising times (TR) of 400s, 600s, and 800s respectively. At temperatures below 100 °C, there was no significant difference between the treated and control samples for quality parameters like acid value, peroxide value and free fatty acid content. However, at 400 and 600 s, significant differences in the chemical quality parameters of the treated samples and the control were shown, where treated samples had some quality deterioration compared to the control. Furthermore, the results

of the same study showed some evidence that PL could affect oil colour. Both a*(redness-greenness) and b* (yellowness-blueness) values increased with time while the L* (lightness) value did not seem to be influenced by the treatments (Abuagela *et al.,* 2018). Therefore, the application of pulsed light can be recommended as a propitious technology to detoxify aflatoxin-contaminated edible oils on a mass scale.

Adsorbents: Activated carbon, (Seifert *et al.,* 2010) kaolinite, clay, (Kang *et al.,* 2016) bentonites (Kong *et al.,* 2014) and grape bagasse (Avantaggiato *et al.,* 2014) are the most common adsorbents used to remove of aflatoxins in food products. Kang *et al* (2014) also have revealed, the adsorption percentage of AFB1 by different adsorbents like activated charcoal, cellulose, a yeast cell walls and bentonite clays as 100%, 13.5%, 92.7%, and 92.5% respectively. Activated carbon and activated charcoal hold the highest places among the most effective adsorbent types. Although they are capable of eliminating a high concentration of aflatoxins from oils by increasing the amount of adsorbent added, oil loss will also pile up.

Consequently, the designing of adsorbent nanomaterials like magnetic graphene oxides (MGOs) with greater power of adsorption has been given priority. Both the ease of separation due to the magnetic field the and high level of removal efficiency to remove aflatoxins from contaminated edible oils have made MGOs an excellent physical separation method. Unlike activated carbon and charcoal, MGOs were capable of separating aflatoxins with minimal oil loss (Xie *et al.,* 2018). However, MGOs may affect the physical and organoleptic properties of edible oils which will need some further studies to prove scientifically. The rare applications of absorbents in the edible oil industry can be due to the loss of nutritional quality (pigments and micronutrients) and loss of the quantity of oil which will cause deterioration of oil quality (Sun *et al.,* 2012).

Chemical Methods

Use of alkalis: Alkaline solutions of NaOH/ KOH can be described as a frequently used approach in removing aflatoxins in the commercial edible oil manufacturing industry (Pankaj *et al.,* 2018). Both the quality and the safety evaluation of chemically treated food is important because there can be chances of mycotoxins to transfer to other compounds present in food and also altering the nutritional composition and flavor profile of the food commodity (Ismail *et al.,* 2018).

Use of electrolyzed water: Electrolyzed water technology can be introduced as a novel chemical technique designed for eliminating aflatoxins in food products (Udomkun *et al.,* 2017). Alkaline electrolyzed water having higher pH and a strong reducing ability was found to be effective in eliminating the majority of AFB1 from edible plant oils contaminated with aflatoxins (Fan *et al.,* 2013). The zero production of chlorine compounds and is a safe procedure to carry out are the key benefits of utilizing this method to get rid of aflatoxins from contaminated foods.

Ozone treatment: Ozone treatment is a certified chemical method (GRAS) used in the food industry. Recent research work has demonstrated that ozone treatment (concentration - 6.0 mg/l, duration - 30 min) for peanuts could degrade total aflatoxins and particularly AFB1 levels up to around 66%. Furthermore, the results indicated, that complete degradation of aflatoxins was achieved, accordingly could reduce cancer risks in tested mice (Isikber *et al.,* 2015).

Biological Methods

These methods involve the use of biological agents to detoxify aflatoxins present in food crops. It can be achieved through two basic methods such as enzymatic degradation and absorption (Jard *et al.,* 2011). Aflatoxins can be absorbed directly by microorganisms either via concatenating to their cell wall contents (Motawe *et al.,* 2014) or by absorbing into dead

microorganisms (Mwakinyali *et al.,* 2019). Similarly, decomposition of aflatoxins can also be fulfilled via administering intra or extracellular enzymes to them, which eventually ended up with CO_2 and H_2O, which are relatively non-toxic or less toxic to the environment (Aliabadi *et al.,* 2013). Previous literature reported that 74.3% to 99.9% of aflatoxin reduction efficiency could be achieved when toxigenic microbial strains of *A. parasiticus* and *A. flavus* were administered to peanut-grown soil. (Dorner *et al.,* 1998). Since no studies have been carried out to investigate the feasibility of the application of biological methods in the edible plant oil industry, the authors would like to suggest it as a great piece of novel research idea for new research and would be a good opportunity for the coconut oil industry as well if explored.

Legislations for Aflatoxins in Edible Plant Oils

Minimizing the toxigenic fungal growth on copra by applying proper drying and storage practices of raw materials are major factors that contribute to the exclusion of aflatoxin contamination in coconut oil. Hence, the coconut oil industry should take steps to minimize possible aflatoxin contamination to ensure the safety of oil which would positively benefit both the industry and the consumer. In this aspect establishment of permissible maximum recommended levels for aflatoxin for a particular edible oil is significant. At present, there are several established maximum permissible levels for aflatoxins in human food and animal feed commodities that can be observed by more than 100 countries through several standard establishment organizations (Bordin *et al.,* 2014; Karunarathna *et al.,* 2019). One of such bodies which establishes standards includes Codex which has established maximum permissible levels for aflatoxins in both human and animal food (Van, 1989; Dissanayake and Manage, 2009; Wu and Guclu, 2012; Smith, 2020). Lately, the national standards establishment organization in Sri Lanka known as Sri Lanka Standards Institute (SLSI), has taken steps to include maximum permissible levels for aflatoxins in their certification scheme for edible oils. However, it is not compulsory for edible oil producers in Sri Lanka to comply with these rules as participation in SLS certification is completely voluntary. As per the European Union (EU), the maximum permissible limit for total aflatoxins is 4 µg/kg and AFB_1 is 2 µg/kg applicable to vegetable oils (Karunarathna *et al.,* 2019; Vasseghian *et al.,* 2020).

The records in (Table 03) reasonably provide an indirect indication for the development of setting levels of aflatoxin limits in the coconut oil industry, which may have occurred due to lack of awareness of the consumers as well as industrialists about the potential negative health concerns of aflatoxins.

CONCLUSIONS

Aflatoxin contamination in edible plant oils is one of the major controversial issues that the food industry faces, owing to their acute and chronic toxicological effects on consumers. Therefore, a considerably huge attention is drawn towards eliminating or transforming them to less toxic by-products and reducing their bioavailability in the final oil product to ensure consumer safety. In addition, the bio-accessibility of aflatoxin is the fraction of aflatoxins released from the oil in the gastrointestinal tract which become available for intestinal absorption (Raiola *et al.,* 2012) and may depend on many factors.

Even though complete decontamination of aflatoxins from edible oils seems impractical, reducing the concentrations below maximum permissible levels is possible. However, the use of chemical agents like alkali solutions is the most commonly practiced method of decontaminating aflatoxins at commercial scale, the environmental impact and the consequences of their consistent usage to human health is questionable and need further investigation. The technique of UV irradiation seems to be a promising physical method of detoxification of aflatoxins in oil, if it can be used combined with

other potential methods such as use of adsorbents. However, the use of biological methods in edible plant oil industry requires further efficacy and safety studies. Care should be taken whatever the technique we apply, it should be able to significantly reduce aflatoxins in oil with no substantial changes to nutritional, sensory as well as quality attributes of the final detoxified oil product to preserve food safety. Further research is required to investigate the potential application of the proposed quantification and decontamination methods in the coconut oil industry.

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Conflicts of Interest

The authors declare that they have no conflicts of interest in relation to this article.

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