Contemporary Feature

Aflatoxins

K. Sarath D. Perera and A. D. Theeshya Dulmini
Department of Chemistry, The Open University of Sri Lanka

Nowadays, "Aflatoxin and Coconut oil" has become a provocative topic in Sri Lanka. However, **aflatoxins (AFs)** were first discovered in England, around 1960s, after the outbreak of turkey X disease. In 2012, the International Agency for Research on Cancer (IARC) categorized aflatoxins (B_1, B_2, G_1, G_2 and M_1) as a "Group 1" **mycotoxin**, which means that they are **carcinogenic** to humans.

What are mycotoxins?

All living organisms inherit their own, natural, self-defense system. Mycotoxins are toxic secondary metabolites produced by fungi, which readily colonize on substrates. Secondary metabolites (that are not directly involved in the growth, development, or reproduction) of plants, bacteria and fungi often play specific and important roles for their survival/existence in the natural habitat. Bacteria produce endotoxins and exotoxins whilst molds (fungi) produce mycotoxins. Bacterial toxins are composed of proteins which destroy a specific target species or host cell, and toxins produced by molds are comprised of simple low molecular weight compounds. There are various types of mycotoxins: aflatoxins, ochratoxins, citrinin, ergot alkaloids, *etc.*

Genus *Aspergillus*

Aflatoxins are mainly produced by fungi belonging to a genus named *Aspergillus*. The separated hyphae which appear as branched filaments make up the mycelium structure of this fungus (Figure 1).

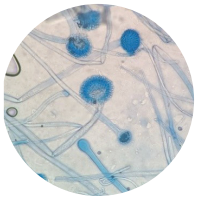


Figure 1: Mycelium of *A. flavus*

The end of the rough and colorless conidiophore (the hypha that bears conidia) swells to make the vesicle which bears the phialids (Figure 2). These phialids can be arranged in either one row (uniseriate) or two rows (biseriate). Conidiophores are asexual spores that are observed at the end of the phialids. Their mycelia excrete various chemical compounds, as they are saprophytic organisms. *Aspergillus flavus* and *Aspergillus parasiticus* are the main causative microbes that produce aflatoxins.

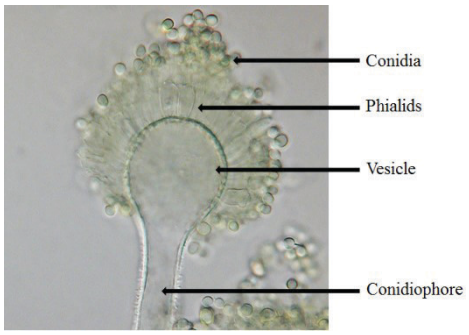


Figure 2: Morphology of *A. flavus*

Their colonies can be visualized as **green** (dark green - *A. parasiticus*, light green - *A. flavus*) velvet mats (Figures 3 and 4).

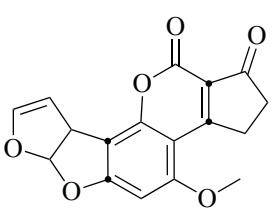
Figure 3: Colony of *A. flavus*

Figure 4: Colony of *A. parasiticus*

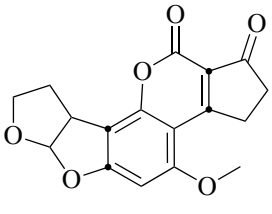
Chemical nature of aflatoxins

Aflatoxins (AFs) are stable polycyclic compounds, belonging to the **difurano coumarin** compounds and are resistant to roasting, extrusion and cooking. There are at least 20 types of AFs found in nature. AFB_1 is the most

toxic compound that is produced by both *A. flavus* and *A. parasiticus*. *A. flavus* produces B-AFs, while *A. parasiticus* is responsible for the production of both type B-AFs and G-AFs. In nomenclature, English letters (**B** and **G**) stands for the **fluorescence color** under UV radiation (B for **blue** and G for **green**) and the number represents the relative chromatographic mobility.

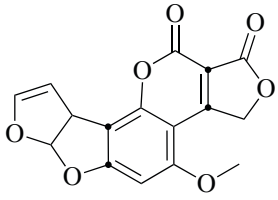


AFB_1

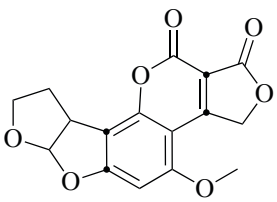


AFB_2

In both AFB_1 and AFG_1, there is a double bond between carbon atoms in the furan ring which allows them to form epoxides while, AFB_2 and AFG_2 lack that functional group.

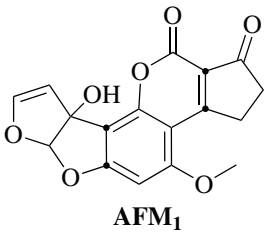


AFG_1

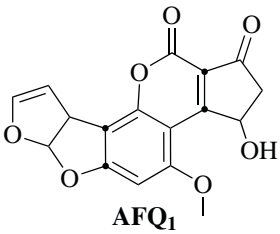


AFG_2

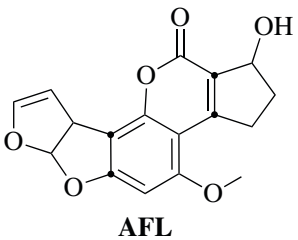
AFM and AFQ are metabolites of AFB types. For example, AFM_1 is a metabolite of AFB_1, in humans and animals and AFB_2 metabolizes to produce AFM_2, in milk of cattle fed on contaminated foods. AFQ_1 is another metabolite of AFB_1, which is prepared in the liver of other higher vertebrates. Aflatoxicol (AFL) is a reductive metabolite of AFB_1.



AFM_1



AFQ_1



AFL

A. flavus is also composed of other mycotoxins, such as sterigmatocystin, cyclopiazonic acid, kojic acid, glycotoxin, aspertoxin, *etc*.

Toxicity

AFs are acutely toxic, immunosuppressive, hepatotoxic, mutagenic, teratogenic (causing birth defects on an embryo and fetus) and carcinogenic compounds. AFs do not have any color, odor, or flavor. Therefore, it is easy to consume such contaminated food unknowingly. Ground nuts, maize, rice, cereals, dried foods, spices, crude vegetable oils and tree nuts can get easily contaminated, thus the *Aspergillus sp.* are saprophytic microbes. The contamination can occur in any stage of food processing, during pre-harvesting, harvesting, post-harvesting, storing, transporting, and consuming.

According to a recent review (Toxins, 2021), India and Sri Lanka are the leading countries that consume rice with a high content of AFB_1. Furthermore, it stated the maximum permissible limits for AFT (total) in food in the following countries as Singapore (5 μg/kg), Japan, Vietnam, Kenya, South Africa (10 μg/kg), Canada, Malaysia, Korea, Australia, Zimbabwe, Taiwan (15 μg/kg), US, Thailand, Philippine, Hong Kong, Brazil, Nigeria (20 μg/kg), and Sri Lanka, India (30 μg/kg).

According to the Health Ministry of Sri Lanka, the maximum permissible limit of AFs is 30 μg/kg (or ppb; parts per billion). However, Sri Lanka Standard states: - a product shall not exceed the level 5.0 μg/kg for aflatoxin B_1 and 10.0 μg/kg for total aflatoxins (AFT) when determined according to the method given in SLS 962. Acute exposure to a high dose of AFs causes vomiting, abdominal pain and even death. Global Cancer Observatory stated that chronic exposure to lower doses may lead to liver cancer which is the 6th most common killer disease among all ages. The livestock may also get contaminated by consuming the feeds containing AFs.

Methods of detoxification

Low ventilation, ambient temperature and high moisture content are the best conditions to enhance the levels of AFs in food. Insects and other microbes, storage time, spore infection density, suitability of the fungal substrate also affect the contamination. Washing, drying

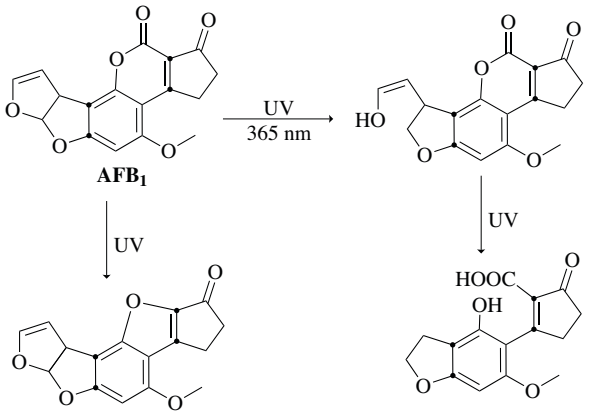
and irradiation are the basic physical methods that can be practiced lowering the effect of AFs. Applying correct fungicides during a specific stage could minimize the contamination. But most of the time, these synthetic fungicides may lead to other adverse ecological and health problems.

The growth of AFs can be inhibited by adding essential oils (EOs). EO contains volatile compounds such as terpenes; they are naturally occurring secondary metabolites of some plants. These chemicals can degrade the cell wall of the fungi and alter the permeability of the membranes. EOs of apple-mint, anise, holy basil, betel, cinnamon, cardamom, lemon grass, coriander, cumin, turmeric, fennel, mint *etc.* can inhibit the effect of AFs. However, it is not practicable to apply EOs to protect foods from AFs, since they are very expensive as well as highly volatile.

As a bio control towards aflatoxins, nontoxic *A. flavus* strain AF36 is currently being used in Arizona, Texas, and Southern California, to substantially reduce the aflatoxin contamination in cottonseed. These strains compete with the toxic *A. flavus* strain and prevent the production of AFs.

AFs are soluble in organic solvents (*e.g.* chloroform and methanol) and slightly soluble in water. Detoxification can be carried out by degrading the structure of AFs. By applying different gases (*e.g.* ozone) or chemical agents (*e.g.* hydrogen peroxide, bases or acids), the chemical nature of AFs can be altered to make them harmless. Main drawbacks of these methods are the high cost and low aesthetic quality of treated foods.

AFs are destroyed when the sample is heated above 250 ^{0}C, but these elevated temperatures are intolerable to many food samples. Due to the photosensitivity of the AFs, the structure of them (especially more toxic AFB_1) can be degraded by sunlight or irradiating the sample with high energy photons. If 2 mm of the coconut oil layer is exposed to sunlight for 10 minutes, 75% of AFs that are present in that sample degrade due to the UV radiation of the sunlight. UV lamps with 365 nm wavelength and 6.4 mW intensity can also be used for this purpose. Gamma radiation (> 3 k Gy) is not very suitable to apply for food samples since it lowers the natural quality of the sample, even though it destroys AFs completely.



TiO_2 is an excellent low cost, eco-friendly photocatalyst, which can be used to degrade AFs. Bentonite, hydrated sodium calcium aluminosilicate (HSCAS), activated charcoal, grape pulp and shell, kaolinite clay are used as AFs absorption agents. Activated charcoal shows the maximum AFs absorption percentage (100%), while, bentonite clay shows 92.5% absorption. Nano materials such as magnetic graphene oxides (MGO) show high absorbance percentages for AFs.

The application of enzymes and microorganisms (yeast strains and lactic acid bacteria such as *Lactobacillus*) are the most convenient and safest methods to reduce AFs in foods (*i.e.* yeast cell wall can absorb about 92.7% AFs in a substrate).

Controversy of coconut oil

Coconut oil is one of major lipid sources used in Sri Lanka. Pure coconut oil is free from cholesterol and shows immense medicinal values. However, the true quality of this coconut oil is masked by adding other oils such as palm oil. The seed of African oil palm or *Elaeis guineensis* is rich in palm oil and its oil can be easily mixed with coconut oil. This adulteration can cause the elevation of AFs, as these nuts can get easily contaminated with *Aspergillus* molds. The other ecological problem caused by this palm plant is that it absorbs an excessive amount of water present in the soil. Therefore, the underground water level decreases, and other plants could die.

Detection of aflatoxins

Thin Layer Chromatography (TLC); Gas Chromatography (GC); High Pressure Liquid

Chromatography (HPLC); Enzyme-Linked Immunosorbent Assay (ELISA), Liquid Chromatography coupled to Mass Spectrometry (LC-MS); Immunoaffinity Column Assay (ICA) are the analytical methods that can be used to detect the AFs. ELISA is useful as it helps to find out whether the antibodies that are related to certain infectious conditions are present in the blood.

In conclusion, the maximum permissible levels of AFs for human consumption vary from 5 to 30 μg/kg (depending on the food type). In Europe, the maximum limit for AFT is 15 μg/kg and for AFB_1, 12 μg/kg. According to the standards of SLSI, the maximum level for AFs in coconut oil is considered as 10 μg/kg.

The health risks from AFs cannot be ignored as it can have a negative impact on human population. However, good practices during (food processing, pre-harvesting, harvesting, post-harvesting, storing, transporting) and public awareness are the best ways to avoid the intoxication of the society by AFs.

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Professor K Sarath D Perera *obtained his BSc from University of Sri Jayewardenepura and completed his PhD in Queen's University Belfast, UK. He is currently serving as a Senior Professor at the Department of Chemistry, Open University of Sri Lanka.*

Ms. A. D. Theeshya Dulmini *graduated from the Open University of Sri Lanka and is currently studying for her MSc. in Analytical Chemistry at the University of Colombo.*

Contemporary Feature

The Biochemistry and Cell Biology Behind Developing Vaccines Against SARS-CoV2 Virus

Ann Sanoji Samarakkody

Department of Pediatric Hematology-Oncology, Boston Children's Hospital

Dana Farber Cancer Institute/Harvard Medical School, Boston, USA.

The beta-Corona virus SARS-CoV-2 has caused a pandemic outbreak that has lasted for over a year and it still continues its impact in a global scale. The devastating loss of lives and livelihoods has caused socio-economic downfall across the world. The scientific community has since worked towards finding therapeutic strategies to mitigate adverse effects of the infection and vaccines to prevent the spread of the virus. The advancements and success in vaccine technologies have been tremendous and has led to the development of effective vaccine candidates under a remarkable time frame.

Pandemics and worldwide spread of viruses over the past several decades had prompted scientists to investigate more efficient alternative vaccine technologies. Beta coronaviruses have caused three outbreaks within the past 20 years: SARS-CoV (2002-2003), MERS-CoV (2012) and lastly SARS-CoV-2 (2019 till present). Since its emergence, SARS-CoV has mutated into few other strains that hold or surpass the high infection rate of the virus originated in Wuhan, China.

1. Biochemical features of SARS-CoV-2

Structurally, SARS-CoV-2 is a pleomorphic virus with a diameter between 50-200 nm. The whole viral genome is encoded in a positive-sense ssRNA, that carries a 5' cap structure and is polyadenylated at the 3' end. The polyadenylation of the genome and as well as the subgenomic mRNAs allow isolation of these molecules using oligo dTs. The whole viral genome of about 29.9 kB contains 5' and 3' untranslated regions of about 200-500 nucleotides. There are 14 open reading frames (ORFs) on the mRNA that transcribe 27 different viral proteins. Among these, non-structural proteins perform essential functions including genome unwinding, replication, capping, tailing, methylation, membrane rearrangement, etc. The structural proteins make the envelop, nucleocapsid, membrane and spike proteins. The spike protein (S) is a multifunctional trimeric transmembrane glycoprotein that plays essential roles is attachment, fusion and entry into the host cell. The S protein is where the initial mutation occurred that led to an outbreak and an eventual pandemic. The cleavage of the S protein trimer is a crucial step in the process of the viral infection. Sequence analysis of the virus had revealed insertion of 4 amino acids between S1 and S2 sub-units of the spike protein. These mutations introduce a new furin cleavage site on the S protein, that was absent in previous SARS-CoV and may have presumably led to the increased pathogenicity of the virus.

The SARS-CoV S trimer has two distinct conformations as shown by Cryo-electron micrography. The change in conformation between open and close forms, is believed to be crucial in its interaction with the human ACE2 (Angiotensin converting enzyme 2) receptors on the cell surface. The opened conformation presents the three hACE-2 recognition motifs on each of the sub-unit and renders it for interaction. ACE2 receptors are highly expressed in Type II alveolar epithelial cells, that explains the respiratory distress associated with the infection. In addition, these S proteins have an abundance of N-linked glycans that protrude out of the viral particle that play roles in protein folding, priming by host proteases and antibody recognition. Sequence analyses confirm the conservation of 20 glycosylation sequences across all SARS S proteins suggesting that