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Aflatoxin B1 in Rice Marketed in Lebanon: Occurrence and Exposure Level

By

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Dedication Page

To my beloved parents

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Aflatoxin B1 in Rice Marketed in Lebanon: Occurrence and Exposure Level

Rita Kordahi

ABSTRACT

Rice is one of the world's most staple food products. Being cultivated in subtropical and tropical hot and humid areas, *A. flavus*, *A. parasiticus*, and *A. nomius* fungi can contaminate rice and produce mycotoxins including the highly hepatotoxic and carcinogenic aflatoxin B1 (AFB1). Our study aimed to evaluate the AFB1 levels in packed rice marketed in Lebanon and determine the exposure to this toxin from the rice consumption. A total of 105 packed white, parboiled and brown rice bags were collected, among which 86 were from 43 brands collected during fall and spring. The enzyme-linked immunosorbent assay (ELISA) method was used to measure AFB1. A comprehensive food frequency questionnaire was filled by 200 participants to determine the patterns of rice consumption and subsequently the exposure levels to AFB1 from the rice consumption in Lebanon. AFB1 was detected in 105 out of 105 (100%) of the rice samples. The average concentration ± standard deviation of AFB1 was 0.5 ± 0.3 μg/kg. Contamination ranged between 0.06 and 2.08 μg/kg. Moisture content in all rice samples was below the limit (14%). Only 1% of the samples had an AFB1 level above the EU limit (2 μg/kg). Brown rice had a significantly higher AFB1 level than white and parboiled rice ($p= 0.02$), while a significant difference was found between both collections for the same brands ($p= 0.016$). Packing season, packing country, country of origin, presence of a food safety management certification, grain size, and time between packing and purchasing had no significant effect on AFB1 in rice. Exposure to AFB1 from rice consumption in Lebanon was calculated as 0.1-2 ng/kg bodyweight/day, resulting in 0.05-1.7 additional cancer cases/1,000,000 persons/year. Future studies should assess AFB1 in unpacked rice and routine monitoring must be carried out to take into account smuggled and emerging brands in the Lebanese market.

Keywords: Rice, Aflatoxin B1, Enzyme-linked immunosorbent assay (ELISA), Exposure level, Lebanon.

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List of Abbreviations

AF: Aflatoxin

AFB1: Aflatoxin B1

FAO: Food and Agriculture Organization

RASFF: Rapid Alert System for Food and Feed

EU: European Union

A.: *Aspergillus*

a_w**:** Water activity

HCC: Hepatocellular Carcinoma

IARC: International Agency for Research on Cancer

PMTDI: Provisional Maximum Tolerable Daily Intake

MRLs: Maximum Residual Limits

AFt: Aflatoxin total

MENA: Middle East and North Africa

HPLC: High-Performance Liquid Chromatography

HPLC-FD: High-Performance Liquid Chromatography with Fluorescence Detection

LC-MS/MS: Liquid Chromatography Tandem Mass Spectrometry

TLC: Thin-Layer Chromatography

ELISA: Enzyme-Linked Immunosorbent Assay

Nd: Not detected

HPLC-UV: High-Performance Liquid Chromatography with Ultra-Violet spectroscopy

LC-FD: Liquid Chromatography with Fluorescence Detection

IAC: Immunoaffinity Chromatography

DLLME: Dispersive Liquid-Liquid Microextraction

HPTLC: High-Performance Thin-Layer Chromatography

UHPLC: Ultra-High Performance Liquid Chromatography

TOF-MS: Time-of-Flight Mass Spectrometry

LOD: Limit of Detection

LOQ: Limit of Quantification

LIBNOR: Lebanese Standards Institution

FSMS: Food Safety Management System

HACCP: Hazard Analysis and Critical Control Point

ISO: International Organization for Standardization

BRC: British Retail Consortium

SQF: Safe Quality Food

FSCC 22000: Foundation for Food Safety Systems Certification 22000

IFS: International Featured Standard

CHAPTER 1
LITERATURE REVIEW

1.1 MYCOTOXINS

1.1.1 DEFINITION

Mycotoxins are toxic secondary metabolites that have chemical and thermal stability and minimal molecular weight. They are produced by fungi such as *Aspergillus, Penicillium*, *Alternaria, Fusarium*, and *Claviceps genus* (Ünüsan, 2019). The number of reported mycotoxins exceeds 400; however, the most significant species in terms of food safety and economic assessment are Aflatoxins (AFs) which are produced by *Aspergillus*, ochratoxin A (OTA) which is produced by *Aspergillus* and *Penicillium*, trichothecenes (TCTs) [Type A: HT-2, T2 toxin and diacetoxyscirpenol (DAS); type B: deoxynivalenol (DON) and nivalenol (NIV); type C &D] which are produced by *Fusarium*, zearalenone (ZEN) which is produced by *Fusarium*, fumonisins B1 and B2 (FBs) which are produced by *Fusarium*, and finally patulin (PAT) which is produced by *Aspergillus*, *Penicillium, Paecilomyces, Eupenicillium* and *Byssochlamys* (Shi et al., 2018).

In the agricultural field, several plant crops, such as cereals, nuts, forages, fruits, vegetables and their by-products, present a high risk of contamination with mycotoxins, in addition to animal products such as meat, eggs, and milk in case the consumed animal feed is contaminated with the toxins (Shi et al., 2018). The Food and Agriculture Organization (FAO) reported that mycotoxins have contaminated around 25% of food crops worldwide (FAO, 2013). Moreover, the Rapid Alert System for Food and Feed (RASFF) of the European Union (EU) ranks mycotoxins in the second position when identifying the total number of hazard notifications (RASFF, 2017).

1.1.2 FACTORS LEADING TO MYCOTOXIN PRODUCTION

According to CODEX Alimentarius, multiple environmental conditions affect the production of mycotoxins in crops during the several steps of the food chain: pre-harvest, harvest and drying, and storage (CODEX Alimentarius, 2012). Incorrect agricultural and harvesting steps, erroneous drying, handling, packaging, storage, and transport procedures

enhance the development of fungi leading to an increased probability of mycotoxins production (Marin, Ramos, Cano-Sancho, & Sanchis, 2013; Marroquín-Cardona, Johnson, Phillips, & Hayes, 2014). High water activities (a_w) between 0.80 and 0.99, and elevated temperatures between 25 and 30°C, enhance the growth of fungi, and subsequently increase the risk of mycotoxins presence especially in staple foods where it can produce synergistic harmful outcomes (Ortiz, Van Camp, Mestdagh, Donoso, & De Meulenaer, 2013). On the other hand, drought causes major stress in the plants by decreasing their natural immunity to fight pathogens and increasing the production of reactive oxygen species which are crucial for mycotoxins production (Marin, Ramos, Cano-Sancho, & Sanchis, 2013).

Subsequent mycotoxin production is hard to occur after food processing when food safety and quality control measures are applied, since these prevent fungal contamination and mycotoxin production (Marin, Ramos, Cano-Sancho, & Sanchis, 2013). There is still controversy around pesticides usage against mycotoxin since data indicated that it may increase or decrease the amount of mycotoxins, depending on the applied level of pesticides. Any kind of mechanical damage to the seeds and grains or its contact with the soil encourages mycotoxins production. (Marroquín-Cardona, Johnson, Phillips, & Hayes, 2014).

Finally, microenvironmental and intrinsic factors from the fungi and seeds also affect fungal growth and vary according to strain specificity and presence of substrates for the development of the mold. Also, several epigenetic and environmentally activated genes have been identified for several mycotoxins, which highlights on the importance of the genome role in mycotoxin production (Marroquín-Cardona, Johnson, Phillips, & Hayes, 2014).

In order to prevent the secretion of mycotoxins, it is crucial to follow the food safety and quality control conditions, including good agricultural practices and good manufacturing practices (Ünüsan, 2019).

1.2 AFLATOXINS (AFS)

1.2.1 HISTORY OF AFLATOXIN

During the year 1960, the epidemic of "Turkey X disease" leaded to the first discovery of AFs when more than 100,000 turkeys in England became sick and died within several months. Symptoms of high poisoning and death of the animals was followed shortly. Severe intestinal inflammation and liver necrosis were proved to be present for the turkeys that went through a post-mortem analysis. Later on, it was revealed that the cause was due to the groundnut meal from Brazil which was consumed by the turkeys and was proven to be highly toxic when fed to poultry during trials. Moreover, the toxins of *Aspergillus flavus,* that were then known as aflatoxins (*A. flavus* toxins), were discovered to be the main reason causing this incident (Rushing & Selim, 2019; Pitt & Miller, 2016).

1.2.2 DEFINITION

AFs are toxic secondary metabolites produced by the fungal species, mainly by *Aspergillus flavus*, *A. parasiticus* and *A. nomius* (Saha Turna & Wu, 2019). AFs are categorized into two subgroups: difuranocoumarocyclopentenones consisting of AFB1, AFB2, AFM1 and AFM2, and the difuranocoumarolactones consisting of AFG1 and AFG2. *A. flavus* species only produce AFB1 and AFB2, while *A. parasiticus* produce AFB1, AFB2, AFG1 and AFG2 (Ünüsan, 2019). *A. flavus* is ubiquitous and can colonize several oil-rich plant crops, such as maize, peanuts and cottonseeds during pre-harvest and post-harvest (Shi et al., 2018). However, *A. parasiticus* prefers soil area and has a limited allocation (Marin, Ramos, Cano-Sancho, & Sanchis, 2013). The fungal genera that produce AFs require temperatures of 25–37°C and moisture of 80–85% for growth (Nazhand, Durazzo, Lucarini, Souto, & Santini, 2020).

However, the production of AF requires a narrower range of conditions compared to fungal growth. It has been shown that the optimum temperature and a_w for the growth of *A. flavus/ parasiticus* were 35°C and 0.95, respectively. On the other hand, the optimum temperature and a_w for AF production by these fungal genera were 33°C and 0.99, respectively (Mannaa & Kim, 2017). Sorenson *et al*. demonstrated that the optimum temperature for AF production by *A. flavus* on rice grains was 28°C. AF production was still apparent at 32°C (Sorenson, Hesseltine, & Shotwell, 1967).

Out of all the mycotoxins, AFB1 is the most prevalent and has the highest toxicity and carcinogenicity levels (Saha Turna & Wu, 2019). Nevertheless, during cooking and food processing, all AFs, and especially AFB1, become highly stable and thermally resistant (melting point above 250 ºC), creating a challenge for its removal from dried products. AFs also remain stable at pH between 3 and 10. For these reasons, AFs require continuous monitoring (Al-Zoreky & Saleh, 2019).

1.2.3 AFLATOXIN METABOLISM

AFs are liposoluble compounds that undergo absorption in the gastrointestinal and respiratory tracts into the blood stream in order to reach the liver where its main metabolism will occur (Ünüsan, 2019). The human cytochrome P450 (CYP) enzymes that metabolize AFs are CYP3A4, 3A5, 3A7, and 1A2. AFB1, known as the most damaging mycotoxin, is mainly metabolized in the liver into the carcinogen AFB1-8,9-epoxide (AFBO) that has 2 isomers AFB1-8,9-exo-epoxide and 8,9-endo-epoxide. Then, exo-epoxide is linked to DNA to create the predominant 8,9-dihydro-8-(N7-guanyl)-9-hydroxy AFB1 (AFB1- N7-Gua) adduct leading to DNA mutation and higher risk of hepatocellular carcinoma. Furthermore, AFB1-N7-Gua can be transformed into two secondary lesions: apurinic site and a steadier ring opened AFB1-formamidopyrimidine (AFB1-FAPY) adduct which persists more in vivo compared to AFB1-N7-Gua (Marin, Ramos, Cano-Sancho, & Sanchis, 2013; Al-Zoreky & Saleh, 2019).

1.2.4 HEALTH CONSEQUENCES

The Consumption of contaminated food and the respiratory and dermal exposures to mycotoxins lead to “Mycotoxicosis” (Ünüsan, 2019). Toxicity of mycotoxins varies when undergoing metabolism. The toxicity in animals and humans depends on different factors such as species, age, nutrition, length of exposure, etc… Multi-exposure to several metabolites makes it more difficult to assess the toxic health reactions because of the additive, synergic or antagonist adverse effect (Pereira, Fernandes, & Cunha, 2014). However, the rumen microbiota can degrade mycotoxins, which explains the reason of higher toxicity in humans compared to ruminants (Ünüsan, 2019).

AFs are known for their extreme toxicity and adverse effects. Out of all the AFs, AFB1 has the highest toxicity level followed by AFM1, AFG1, AFB2, and AFG2 (Ünüsan, 2019). They are genotoxic, carcinogenic, teratogenic, mutagenic, hepatotoxic and immunosuppressive (Theumer et al., 2018).

The AF toxicity, known as "Aflatoxicosis", can be either acute or chronic. Acute toxicity is rare and happens when high doses of AFs are present in the food. It leads to vomiting, abdominal pain, pulmonary or cerebral oedema, hemorrhage, necrosis, fatty liver, anorexia, depression, jaundice, diarrhea, photosensitivity and sometimes death. (Marin, Ramos, Cano-Sancho, & Sanchis, 2013; Saha Turna & Wu, 2019). Chronic aflatoxicosis is the most occurring form. It is common when small doses of AFs are consumed in food over a long period of time, leading to different human diseases such as:

- **Hepatocellular carcinoma (HCC)**: data have shown that hepatitis B and/or C viruses work in a synergetic manner with AFs in the etiology of HCC (Marin, Ramos, Cano-Sancho, & Sanchis, 2013).
- **Reproductive system effects:** AFs can influence negatively the reproductive male system by affecting the development and morphology of the testis, diminishing the reproductive capacity, sperm count, and testosterone levels (Marin, Ramos, Cano-Sancho, & Sanchis, 2013).
- **Immune system effects:** AFs contamination can play the role of immunomodulators causing dysfunction and suppression of humoral and cell mediated immunity adding to it inflammation promotion (impaired T or B lymphocyte work, decreased macrophage/neutrophil functions, etc…) (Marroquín-Cardona, Johnson, Phillips, & Hayes, 2014). Furthermore, secondary infections by fungi, bacteria, and parasites will become more resistant along with a reduced immunity to vaccines (Marin, Ramos, Cano-Sancho, & Sanchis, 2013; Saha Turna & Wu, 2019).
- **Encephalopathy along with fatty degeneration of viscera** as similar as Reye's syndrome (enlarged liver and kidneys, edema, stroke…). The role is still controversial (Marin, Ramos, Cano-Sancho, & Sanchis, 2013).

- **Pulmonary interstitial fibrosis**: The risk increases when the AF passes through the respiratory tract (Marin, Ramos, Cano-Sancho, & Sanchis, 2013).
- **Malnutrition and growth suppression:** Growth impairment and malnutrition occur after the harm caused by AFB1 to the intestines and liver (Marroquín-Cardona, Johnson, Phillips, & Hayes, 2014). AFB1 disrupts the intestinal epithelial cells leading to breakdown of the intestinal structure. Moreover, the inflammation takes part by increasing the immune cells which might harm the tissue and decrease the capacity of nutrients' absorption (i.e. vitamins A and E) and receptors (i.e. Vitamin D receptor). Nonetheless, AFB1 can also disrupt the distribution of nutrients to organs (i.e. metals) (Rushing & Selim, 2019). Finally, the AFB1 liver toxicity can affect the growth hormone and insulin-like growth factor signaling axis causing a regression in bone and tissue development, especially in children that will suffer from delayed growth (Mottaghianpour, Nazari, Mehrasbi, & Hosseini, 2017; Rushing & Selim, 2019).

Moreover, the International Agency for Research on Cancer (IARC) classified AFB1, AFB2, AFG1, and AFG2 as group 1 carcinogen and AFM1 as group 2B carcinogen. When assessing the risk of AFB1 food contamination, a provisional maximum tolerable daily intake (PMTDI) of 1 ng AFB1 per kg body weight per day is adopted for adults and children not infected with hepatitis B, and 0.4 ng AFB1 per kg body weight per day is adopted for adults infected with hepatitis B (Ali, 2019). Nonetheless, AFs are the only metabolites that are being monitored by US Food and Drug Administration action levels while the rest of mycotoxins are only disposed to consultative levels (Ünüsan, 2019). Also, the EU created legal limits between 4 and 15 μg kg^{-1} for AFs in different foods (European Commission, 1881/ 2006).

1.3 RICE

1.3.1 RICE VARIETIES

Rice is one of the world's most important staple foods. Its consumption comes after wheat (Ali, 2019; Ok et al., 2014). According to FAO, rice represents 27% of the global energy uptake and 20% of the dietary protein in the developing countries (FAO, 2004).

There are more than 40,000 varieties of rice cultivated in the world. However, there are two predominant species cultivated widely: Oryza sativa as known as the Asian rice and Oryza glaberrima as known as the African rice. Oryza sativa is widely cultivated, unlike Oryza glaberrima, which is only cultivated in Africa. Oryza sativa is characterized by having two major subspecies: The Indica, long-grain rice and the Japonica, short round-grain rice. The Japonica rice is mostly cultivated and consumed in Australia, China, Taiwan, Korea, the European Union, Japan, Russia, Turkey and the USA, while the Indica rice species are cultivated over a wide range in Asia. These varieties also include some with fragrant characteristics which are priced as premium, such as the Hom Mali from Thailand and the different types of Basmati cultivated in the Himalayan foothills of India (Haryana and Punjab) and Pakistan (Punjab) (Rathna Priya, Eliazer Nelson, Ann Raeboline Lincy, Ravichandran, & Antony, 2019).

The huge variety or rice relies on the difference between cereal length, color, aroma, flavor, stickiness, thickness and development states that affect the quality of the grain. The location and culture impact the type of the worldwide rice market (Fukagawa & Ziska, 2019). Rice is widely categorized based on the shape or method of processing the grains:

1.3.1.1 SHAPE OF THE GRAINS

It indicates the length and width of the grain after cooking:

- Long grains have a slender kernel over four times as long as they are wide. After cooking, the grains remain separate and fluffy (e.g., Jasmine and Basmati rice).
- Medium grains have a shorter, wider kernel. After cooking, the grains remain tender and semi-sticky (e.g., Arborio rice).
- Short grains have a kernel only twice as long as they are wide. After cooking, the texture of the grains remains the stickiest (e.g., "sushi" rice) (Harvard Chan, 2021).

1.3.1.2 PROCESSING AND NUTRITIONAL VALUE OF THE GRAINS

Rice grains have complex matrices containing carbohydrates, proteins, fats, fiber and other micronutrients (Škrbić, Ji, Živančev, Jovanović, & Jie, 2017). After harvesting, rough rice (paddy rice) will be dried and kept in store from few months to several years

(Al-Zoreky & Saleh, 2019). Rough rice is comprised of the hull, an outer protective layer, and the fruit or rice caryopsis (brown or dehusked rice). Afterwards, rough rice will be de-hulled in order to obtain brown rice which comprises the following outer layers: the pericarp, seed-coat and nucellus; the germ or embryo; and the endosperm. (Rathna Priya, Eliazer Nelson, Ann Raeboline Lincy, Ravichandran, & Antony, 2019).

Nevertheless, parboiled rice is formed after soaking in water, steaming under pressure, drying and cleaning the paddy or dehusked rice prior to milling. This processing method hardens the grain by fully gelatinizing the starch and decreases the probability of overcooking, and helps retaining a high level of the natural vitamin and mineral composition found in the milled layers (Al-Zoreky & Saleh, 2019; Morrison, Ledoux, Chester, & Samuels, 2019). Moreover, brown rice can undergo polishing (milling) that eliminates the germ (embryo) and the bran layer of the rice in order to yield white rice. Milling leads to the loss of fat, protein, B vitamins, phytochemicals (polyphenols, anthocyanins and flavonoids), phosphorus, calcium along with tocopherol, tocotrienol, amino acids, γ-oryzanol and fibers that are significantly found in the bran part while enhancing the starchy part in the endosperm, that becomes its predominant macronutrient (Fukagawa & Ziska, 2019). Therefore, brown rice has more health benefits than white rice, with regard to vitamin E for example (Lee, Sreenivasulu, Hamilton, & Kohli, 2019). The content of total dietary fiber in white rice remains lower compared to brown rice after milling (0.7-2% vs. 3-4%, respectively), but the overall caloric content of brown rice is a little bit higher compared to white rice because of the lipid content of the bran (Kaur, Ranawana, & Henry, 2016; Fukagawa & Ziska, 2019). Nevertheless, white rice is rich in minerals such as magnesium, manganese, selenium, iron and phosphorus along with some vitamins such as thiamin, niacin, folic acid. pantothenic acid, folate and vitamin E. However, it does not contain vitamin C, vitamin A, beta- carotene, lutein and zeaxanthin (Rathna Priya, Eliazer Nelson, Ann Raeboline Lincy, Ravichandran, & Antony, 2019).

1.3.2 SOURCES

The cultivation of rice occurs in subtropical and tropical warm and humid areas, such as Asia (Al-Zoreky & Saleh, 2019; Martín Castaño, Medina, & Magan, 2017). Due to its aquatic characteristic, rice is harvested at elevated moisture levels (35-50%)

(Škrbić, Ji, Živančev, Jovanović, & Jie, 2017). Afterwards, the drying procedure takes part. In case the storage circumstances do not respect the food safety measures, rice can get fungal development, leading to the loss of this staple food, and thus to a negative influence on the economy of rice producing areas (Al-Zoreky & Saleh, 2019).

According to FAO, during inconvenient storage circumstances, 15% of cultivated rice is thrown away every year because of the fungi contamination along with other harmful species (Al-Zoreky & Saleh, 2019). Therefore, mycotoxin contamination is an important global food safety concern especially in Asia (Ruadrew, Craft, & Aidoo, 2013). Previous data have shown that out of all the fungal genera, *Fusarium, Alternaria, Penicillium, Rhizopus,* and *Aspergillus* can grow in rice and lead to the production of secondary metabolites such as mycotoxins (Ok et al., 2014).

A. flavus, *A. parasiticus*, and *A. nomius* can produce AFs in rice, including AFB1, AFB2, AFG1 and AFG2 (Ferre, 2016). Several studies performed in many regions of China have revealed that rice is contaminated with many secondary metabolites, especially with AFs (Lai, Liu, Ruan, Zhang, & Liu, 2015). Fourteen % of AFs produced by *A. flavus* and *A. parasiticus* are present in the rice bran and 78% in unpolished rice (Firdous, Ejaz, Aman, & Khan, 2012). Moreover, previous studies have shown that cultivated paddy rice (raw, unprocessed rice), is highly contaminated with *Aspergillus spp* and *A. flavus*, followed by *A. niger* which lead to the formation of AFs in harvested paddy rice. Normally, paddy rice undergoes drying in order to ease the milling step while decreasing the growth of fungi possibility. While undergoing milling, AF levels decrease. However, this reduction relies on technical factors. Brown rice presented higher levels of total AFs compared to white rice. Parboiled rice also contains higher AFs levels (mainly in the bran and husk) compared to raw rough rice (Morrison, Ledoux, Chester, & Samuels, 2019).

Several studies revealed the contamination of rice with AFs in Sri Lanka, Bangladesh, Japan, China, Vietnam, Thailand, India, the Philippines, Korea, United Arab Emirates, Turkey, Tunisia, Nigeria, Cote d'Ivoire, Uruguay, Brazil, Scotland, United States, United Kingdom, Austria, Iran, and Sweden (Bansal et al., 2011; Iqbal, Asi, Hanif, Zuber, & Jinap, 2016).

EU implemented maximum levels of AFB1 and total AFs (2 μg/kg and 4 μg/kg, respectively) in rice for human consumption and also implemented maximum levels of

AFB1 and total AFs (5 μg/kg, 10 μg/kg, respectively) in rice before the ingestion. **Table 1** illustrates the maximum tolerable limit of AF in rice in EU and other countries (Ali, 2019).

Table 1 Maximum residual limits (MRLs) of aflatoxin in rice in EU and other countries

Countries/ Organization	**Aflatoxin**	**MRLs (μg/kg)**
Bosnia and Herzegovina	AFB1	1
Brazil	AFB1	30
Canada	AFt	15
Chile	AFt	5
China	AFB1	10
Egypt	AFt	5
EU	AFB1	2
India	AFt	30
Iran	AFB1	5
Japan	AFB1	10
Korea	AFB1	10
Malaysia	AFt	5
Mexico	AFt	20
Russia	AFB1	5
Switzerland	AFB1	1
Taiwan	AFt	10
Turkey	AFB1	2
USA	AFt	20

EU: European Union; AFt: Aflatoxin total.

1.3.3 PRODUCTION AND CONSUMPTION PATTERNS

It is taught that the first culture of rice occurred in China, India, and Indonesia (Kaur, Ranawana, & Henry, 2016). From 1960 to 2010, the worldwide consumption of rice increased from 156 million tons to 456 million tons (Kaur, Ranawana, & Henry, 2016). According to FAO, the global production of rice is in regular increase and milled rice production reached 501.2 million tonnes in 2016/2017 (FAO, 2018). Moreover, in 2017/2018, the total worldwide milled rice consumption was almost 485 million metric tons (Ali, 2019). Based on the Ricepedia data, more than 90% of production and consumption of rice worldwide takes place in Asia and the current share in global rice consumption is around 87% (Rathna Priya, Eliazer Nelson, Ann Raeboline Lincy, Ravichandran, & Antony, 2019). Most of the rice production in Asia is primarily established by China and India. Nonetheless, the rice consumption is not equal within the countries, for example, the nations with higher urbanization, like Japan, consume 65 kg per capita which is lower by four times compared to an overpopulated country, such as Bangladesh (258 kg) (Milovanovic & Smutka, 2017; Ali, 2019). **Figure 1** illustrates the top ten countries in terms of rice production and consumption (Ali, 2019).

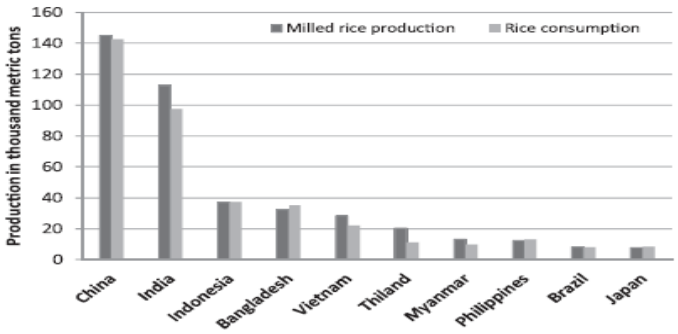


Figure 1 Rice production and consumption in top ten countries in the world in 2017/2018

Asia is the continent with the most elevated rice consumption (IRRI, 2010; Kaur, Ranawana, & Henry, 2016). China ranks the first place in Asia for the highest rice production and the first place globally for rice cultivation space and production yield

(Milovanovic & Smutka, 2017). In 2012, the rice consumption per person reached 67.6 kg/year in China (Lai, Liu, Ruan, Zhang, & Liu, 2015), and, in 2014, 31% of Asia's harvest was reached by China (Milovanovic & Smutka, 2017). Moreover, in 2015/2016, China occupied the first place of the overall annual rice production and accounted for 29.2% of the global milled rice production (Sun, Su, & Shan, 2017).

Furthermore, a comparison of 154 countries in 2017 revealed that Bangladesh ranked the highest country for rice consumption per capita with 269 kg followed by Laos and Cambodia. The countries ranking the end of the scale were Serbia with 0.997 kg, Tunisia with 1.22 kg and Poland with 1.61 kg. Moreover, based on Faostat data in 2017, the average worldwide rice consumption per capita reached 79.9 kg which is 0.271% more compared to the previous year and 33.4% more compared to 10 years ago. Historically, the average rice consumption per capita attained an all-time high of 79.9 kg in 2017 and an all-time low of 38.8 kg in 1961. The average annual growth reached 1.30% since 1961 (Helgi Library, 2021).

Nonetheless, the highest rice producing countries in the Middle East and North Africa (MENA) region are Egypt, Turkey, and Iran. However, the production is way behind the consumption and will not increase due to climate and land limitations. The consumption of the MENA region during 2011-2013 was on average 13 million tons of rice per year out of which about 7 million tons were imported (**Figure 2**) (Nigatu, Motamed, Economic Research Service, United States, & Department of Agriculture, 2015).

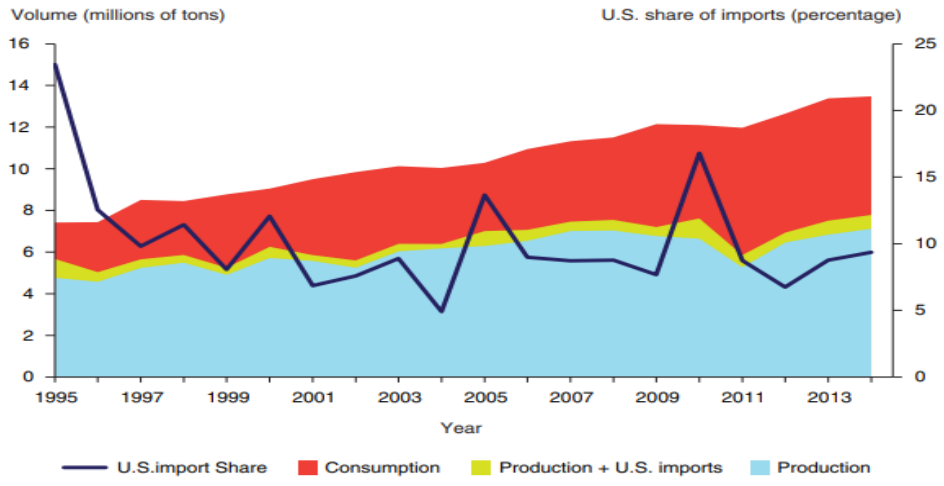


Figure 2 Rice consumption, production, and trade for MENA, and U.S. import share

It is though that the rice production in the MENA region will increase by only 0.5% during the 10-years following 2014. Egypt has the highest rice consumption rate in the region. However, regional demand and export capacity of excess production are adequately covered. Moreover, Iran ranks the second place for rice consumption in the MENA region (Nigatu, Motamed, Economic Research Service, United States, & Department of Agriculture, 2015).

Nonetheless, Iraq and Saudi Arabia are also known for their important rice consumption level. They import even higher amounts of rice as % of consumption compared to Iran and their imports are assumed to increase until 2024 (**Table 2**) (Nigatu, Motamed, Economic Research Service, United States, & Department of Agriculture, 2015).

Table 2 Projected MENA consumption and imports for major crop commodities

	Consumption						Imports		
	Food use			Feed use					
	Average 2012/14	Projected 2024	Growth rate (%) 2013/24	Average 2012/14	Projected 2024	Growth rate (%) 2013/24	Average 2012/14	Projected 2024	Growth rate (%) 2013/24
Wheat									
Egypt	16,300	18,292	1	1,933	1,791	-0.7	9,323	10,545	1.1
Morocco	8,400	9,646	1.3	333	342	0.2	3,575	3,842	0.7
ONA	14,723	17,671	1.7	75	87	1.3	10,618	11,509	0.7
Saudi Arabia	3,133	3,900	2	150	181	1.7	2,867	3,904	2.8
Turkey	16,933	18,052	0.6	617	308	-6.3	4,486	4,868	0.7
Iraq	5,695	7,212	2.1	692	927	2.7	3,398	4,924	3.4
Iran	15,833	16,803	0.5	1,700	1,515	-1	5,633	3,550	-4.2
OME	12,328	14,711	1.6	1,658	1,847	1	10,575	12,426	1.5
Total MENA	**93,347**	**106,287**	**1.2**	**7,158**	**6,998**	**-0.2**	**50,475**	**55,568**	**0.9**
Rice									
Egypt	4,017	4,515	1.1	0	0	0	22	46	6.7
Morocco	56	76	2.7	0	0	0	21	34	4.6
ONA	461	522	1.1	0	0	0	461	522	1.1
Saudi Arabia	1,326	1,602	1.7	0	0	0	1,325	1,600	1.7
Turkey	770	916	1.6	0	0	0	292	383	2.5
Iraq	1,483	1,948	2.5	0	0	0	1,404	1,765	2.1
Iran	3,400	3,880	1.2	0	0	0	1,817	2,112	1.4
OME	1,992	2,357	1.5	0	0	0	1,971	2,360	1.6
Total MENA	***13,505***	***15,815***	***1.4***	***0***	***0***	***0***	***7,312***	***8,821***	***1.7***

OME: Other Middle East; sums Bahrain, West Bank & Gaza, Israel, Jordan, Kuwait, Lebanon, Oman, Syria, United Arab Emirates, and Yemen.

ONA: Other North Africa; sums Tunisia, Libya, and Algeria.

When it comes to the rice consumption per capita of Lebanon, the country has been ranked 85th among 155 countries. The rice consumption per capita was 14.1 kg in 2017 which was 13.2 % lower compared to 2016. In the previous years, the highest rice consumption per capita in Lebanon was 16.2 kg during 2016. When comparing Lebanon

to neighboring countries, in 2017, rice consumption per capita in Cyprus reached 5.09 kg while 19.8 kg in Jordan. (Helgi Library, 2021).

1.4 AFLATOXIN B1 (AFB1) IN RICE

1.4.1 ANALYTICAL METHODS

Many countries have implemented maximum acceptable levels for the presence of AFs in consumed food, which requires sensitive and selective methods for their determination and quantification. High-performance liquid chromatography (HPLC) without or with a fluorescence detection (HPLC-FD), liquid chromatography (LC) coupled to mass spectrometry (MS) detector, thin-layer chromatography (TLC) and enzyme-linked immunosorbent assay (ELISA) are the analytical methods used to quantify AFB1 in rice :(Ali, 2019; Iqbal, Asghar, Ahmed, Khan, & Jamil, 2014).

- **High-Performance Liquid Chromatography (HPLC):** it is known for its high sensitivity, specificity, accuracy, easy usage and reproducibility of results, yet it is expensive and requires skilled personnel (Iqbal, Asghar, Ahmed, Khan, & Jamil, 2014). Derivatisation of AFB1 and AFG1 improves their natural fluorescence in order to make them more detectable and proves the presence of AFs in the sample by HPLC. Therefore, the KOBRA CELL, an electrochemical cell connected to an HLPC system downstream from the HPLC column and in line with the column effluent and the fluorescence detector, has the capacity to overcome the limitations of other derivatisation methods. It creates a reactive form of bromine for derivatisation of AFB1 and AFG1, leading to an improved fluorescence and a more sensitive detection.
- **Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS):** Combining HPLC and MS methods to form LC–MS/MS enhances the chances for better AFB1, resulting in better trace level identification, selectivity, sensitivity, mass spectral portioning and determination of conflicting impurities. However, it is highly expensive and requires highly skilled personnel. It also needs specific sample preparation proceedings (Iqbal, Asghar, Ahmed, Khan, & Jamil, 2014).
- **Thin-Layer Chromatography (TLC):** known for its non-difficult steps, cheap cost, and robustness. More advanced techniques such as HPLC, LC–MS/MS and

ELISA took the place of TLC years ago, but it is still in use. Its disadvantages are inadequate sample clean-up leading to incomplete partitioning, decreased sensitivity and false results (Iqbal, Asghar, Ahmed, Khan, & Jamil, 2014).

- **Enzyme-Linked Immunosorbent Assay (ELISA):** The protocol of ELISA relies on the interaction between antigen–antibody. It allows a highly sensitive and selective quantitative/qualitative analysis of antigens, including plant secondary metabolites. To determine these secondary metabolites, an antigen or antibody is labeled using enzymes. The antigen in the fluid phase is immobilized on the solid phase which is the microtiter plate. Afterwards, the antigen interacts with a particular antibody, that is determined by an enzyme-labeled secondary antibody. The color that appears by using a chromogenic substrate reflects the presence of the antigen. These enzyme–substrate reactions are executed within 30 to 60 min, and the reaction ends by adding a convenient solution. Finally, a microtiter plate reader is used in order to detect the colored or fluorescent products (Sakamoto et al., 2018).

  ELISA is being highlighted as one of the most used techniques for AFB1 analysis because of its easy execution, sensitivity, low cost, adaptability, safety, high-throughput minimal sample extraction and sample volume need. Moreover, the quantitative analysis can be perfectly performed by the intermediate of an available, simple and rapid kit that illustrates comparable results with TLC and HPLC. However, ELISA can present some disadvantages such as difficulty in the quantification of individual AF. It requires as well delicate supervision for each test in order to acquire accurate results (Iqbal, Asghar, Ahmed, Khan, & Jamil, 2014; Pereira, Fernandes, & Cunha, 2014). Nonetheless, there is a high chance of false positive or negative results due to insufficient blocking of the surface of microtiter plate immobilized with antigen and a possibility of antibody instability since the antibody is a protein that requires refrigerated transport and storage (Sakamoto et al., 2018).

1.4.2 REPORTED AFB1 LEVELS IN RICE WORLDWIDE

Since AFB1 has been identified as a major public health concern, plethora of studies throughout the years and all over the world have analyzed AFB1 levels in rice using different methodologies (**Table 3**).

Table 3 Reported AFB1 levels in rice worldwide

Country	**Year of publication**	**Sample size (rice samples)**	**Types of rice**	**Analytical method**	**Reported AFB1 level as µg/kg (mean± SD as µg/kg)**	**Exposure level as ppb or ppm or µg or ng /kg body weight/day**	**Reference**
United Arab Emirates	1999	500	Short and long grain rice	HPLC-UV	1.2-16.5	-	(Osman, Abdelgadir, Moss, & Bener, 1999)
Colombia	2001	40	Rice and rice products	LC-FD	1.0-13.6 (7.1)	-	(Diaz, Perilla, & Rojas, 2001)
Indonesia	2001	2	Rice products	ELISA	2.0-7.0	-	(Noviandi et al., 2001)
Korea	2005	88	Polished rice	HPLC-FD	1.8-7.3 (4.3)	-	(Park, Choi, Hwang, & Kim, 2005)
Philippines	2005	78	Polished and brown rice	IAC, HPLC	ND-8.33 (1.48)	0.1-7.5 ng/kg bw/d	(Sales & Yoshizawa, 2005)
Ivory Coast	2006	10	Rice	ELISA	<1.5-10	-	(Sangare-Tigori et al., 2006)
India	2007	1511	Parboiled rice	HPTLC	<LOD-361	-	(Toteja et al., 2006)
Vietnam	2007	100	Rice	HPLC-FD	nd-29.8 (3.31)	Max: 296 ng/kg bw/day	(Nguyen, Tozlovanu, Tran, & Pfohl-

							Leszkowicz, 2007)
Tunisia	2008	16	Rice	ELISA	Nd	-	(Ghali, Hmaissia-khlifa, Ghorbel, Maaroufi, & Hedili, 2008)
India	2009	1200	Paddy and milled rice	ELISA	0.1-308.0	-	(REDDY, REDDY, & MURALI DHARAN, 2009)
Sweden	2009	99	Basmati, high content of fibre, jasmine and long-grain rice	HPLC-FD, RIDA QUICK	Nd-46.2	2–3 ng/kg bw/ d	(Fredlund et al., 2009)
Austria	2010	81	Basmati, whole grain, long grain, short grain and puffed rice	IAC, HPLC-FD	0.45-9.86	-	(Reiter, Vouk, Böhm, & Razzazi-Fazeli, 2010)
Iran	2010	261	Rice	IAC, HPLC-FD	0.2-4.3 (0.72±0.73)	-	(Feizy, Beheshti, Fahim, Janati, & Davari, 2010)
Turkey	2010	100	Rice	ELISA	ND-1.86 (1.12)	-	(Buyukunal et al., 2010)
Canada	2011	200	White, brown, red, black, basmati, jasmine	IAC-HPLC-FD	Nd-7.1 (0.36)	-	(Bansal et al., 2011)

			and wild rice				
China	2011	29	Rice	ELISA, IAC, HPLC-FD	0.1-1.4 (0.5-0.6)	-	(Sun et al., 2011)
Germany	2011	17	Basmati rice	IAC, HPLC-FD	Nd-4.61 (0.96)	-	(Reinhold & Reinhardt, 2011)
Iran	2011	256	Polished rice	IAC, HPLC-FD	Nd–5.8 (1.4 ±1.0)	1.4–5.8 ng/ kg bw/day	(Rahmani, Soleimany, Hosseini, & Nateghi, 2011)
Malaysia	2011	13	Rice based	ELISA	0.68 - 3.79 (1.75)	-	(Reddy, Farhana, & Salleh, 2011)
Nigeria	2011	21	Rice	TLC, HPLC	4.1-309.0 (37.2±14.0)	-	(Makun, Dutton, Njobeh, Mwanza, & Kabiru, 2011)
Brazil	2012	230	rice with the processing fractions (bran, rice husk and broken)	IAC, HPLC-FD	0.08-180.74 (9.1)	-	(Almeida et al., 2012)
Egypt	2012	40 (samples (1 kg each) of commercial maize and rice seeds	Rice	IAC, HPLC-FD	Nd-19.8	-	(Madbouly, Ibrahim, Sehab, & Abdel-Wahhab, 2012)
Pakistan	2012	519	White, brown and sella rice	HPLC	Range: 2.01- 16.65 Mean overall:	-	(Firdous, Ejaz, Aman, &

					Brown rice: 0.56 White rice: 0.49 Sella rice: 0.73		Khan, 2012)
Banglad esh	2013	2.5 Kg	Milled rice	HPLC	<LOD-0.9 (0.3±0.4)	-	(Roy et al., 2013)
China	2013	31	White, brown, black, red kojic rice	IAC, HPLC-FD	red kojic rice: 2.9	-	(Zhu, Liu, Chen, & Cheng, 2013)
Ecuador	2013	121 (paddy rice) and 125 (polishe d rice)	Paddy and polished rice	IAC, UHPLC/ TOFMS	Paddy rice: 4.9-47.4 (20.6 ±23.3)	-	(Ortiz, Van Camp, Mestdagh, Donoso, & De Meulenaer , 2013)
Iran	2013	65	Domesti c rice	LC-MS/MS	<LOQ-30.83 (3.90)	-	(Nazari, Sulyok, Yazdanpa nah, Kobarfard, & Krska, 2014)
Iran	2013	18	Rice	IAC, HPLC-FD	1.17 30.63 4.17 (9.36 ±)	Mean: 2.29 ng/Kg bw/d Max: 30.63 ng/Kg bw/d	(Yazdanpa nah et al., 2013)
Iran	2013	200	Yellow and white rice	IAC, HPLC	Yellow: 0.01-0.88 White: 0.07- 2.36	-	(Karajiban i, Merkazee, & Montazeri far, 2013)
Mexico and Spain	2013	67	White, Sinaloa, Morelos,	IAC, HPLC-FD	Mexico: < LOD- 8.1 Spain: < LOD-91.7	-	(Suárez-Bonnet et al., 2013)

			Wild, basmati and bomba rice				
Pakistan	2013	68	Brown rice	HPLC-FD	8.23 ± 1.87	-	(Majeed, Iqbal, Asi, & Iqbal, 2013)
Thailand	2013	35	Unpolished and unpolished glutinous rice	IAC, HPLC-FD	Glutinous rice: 0.06-36.64 (18.35)	-	(Tansakul, Limsuwan, Böhm, Hollmann, & Razzazi-Fazeli, 2013)
China	2014	370	Rice	Dispersive liquid liquid microextraction (DLLME) coupled to HPLC-FD.	0.030-20.0 (0.60 ± 2.1)	-	(Lai, Liu, Ruan, Zhang, & Liu, 2015)
China	2014	25g	Polished rice grain	DLLME, HPLC-FD	175-124101 (5884)	-	(Lai, Zhang, Liu, & Liu, 2015)
Pakistan	2014	1025	Super kernel basmati, basmati, parboiled and broken rice	HPLC-UV	Super Kernel basmati: 1.1-32.9 Basmati: 1.0-15.4 Parboiled: 1.1-9.2 Broken: 2.1-25.3	-	(Firdous, Ashfaq, Khan, & Khan, 2014)
Pakistan	2014	120	Brown rice	TLC, IAC, HPLC-FD, IAC, LC–MS/MS, ELISA	TLC: 1.18-10.08 (3.45) HPLC: 0.21-10.54 (3.56)	-	(Iqbal, Asghar, Ahmed, Khan, & Jamil, 2014)

					LC-MS/MS: 0.10-10.88 (3.73)		
Pakistan	2014	262	Brown rice	TLC	1.07-24.65 (3.80)	-	(Asghar, Iqbal, Ahmed, & Khan, 2014)
Iran	2015	40	Tarom rice	ELISA	0.29-2.92	-	(Eslami, Mashak, Heshmati, Shokrzadeh, & Mozaffari Nejad, 2015)
Thailand	2015	240	Brown and color rice	IAC, HPLC-FD	<LOD-26.61	0.80 and 0.12 μg kg^{-1} bw day^{-1}, in period I and II respectively	(Panrapee, Phakpoom, Thanapoom, Nampeung, & Warapa, 2016)
India	2016	18	9 organic and 9 conventional rice	IAC, HPLC-FD	Nd	-	(Baydan et al., 2016)
Pakistan	2016	208	White, brown rice and rice products	HPLC-FD	White rice: LOD-21.3 (7.70 ± 0.89) Brown rice: LOD-19.8 (8.91 ± 1.20) Rice flour: LOD-9.8 (3.51 ± 1.20) Sweet puffed rice balls: LOD-10.2	Mean: Lower bound: 22.2 ng kg^{-1} bw day^{-1} Upper bound: 22.3 ng kg^{-1} bw day^{-1}	(Iqbal, Asi, Hanif, Zuber, & Jinap, 2016)

					(2.90 ± 0.85) Rice cookies: LOD-12.4 (3.18 ± 0.40) Rice sweets: LOD-15.2 (4.10 ± 1.30) Rice noodles: LOD-11.8 (3.60 ± 0.85) Rice bread: LOD-7.4 (2.40 ± 0.43)		
Serbia and China	2017	13	White, glazed and integral rice	UHPLC	Nd	-	(Škrbić, Ji, Živančev, Jovanović, & Jie, 2017)
Pakistan	2018	180	Polished rice of all varieties	LC–MS/MS	<LOD-40.0 (5.84)	South Punjab (mean as ng/kg b.w. day): Children: 4.16 Adults: 4.11 North Punjab (mean as ng/kg b.w. day): Children: 7.48 Adults: 7.21	(Majeed et al., 2018)

HPLC: High-Performance Liquid Chromatography; HPLC-FD: High-Performance Liquid Chromatography with Fluorescence Detection; LC-MS/MS: Liquid Chromatography Tandem Mass Spectrometry; TLC: Thin-Layer Chromatography; ELISA: Enzyme-Linked Immunosorbent Assay; Nd: Not detected; HPLC-UV: High-Performance Liquid Chromatography with Ultra-Violet spectroscopy; LC-FD: Liquid Chromatography with Fluorescence Detection; IAC: Immunoaffinity Chromatography; DLLME: Dispersive Liquid-Liquid Microextraction; HPTLC: High-Performance Thin-Layer Chromatography; UHPLC: Ultra-High Performance Liquid Chromatography; TOF-MS: Time-of-Flight Mass Spectrometry; LOD: Limit of Detection; LOQ: Limit of Quantification

CHAPTER 2

AIMS AND HYPOTHESES

2.1 GAPS IN THE LITERATURE

Until now, no study was performed in Lebanon to assess the safety of packed rice marketed in the country, in terms of AFB1, to determine the exposure levels from the rice consumption and the associated liver cancer risk from this toxin.

2.2 RESEARCH OBJECTIVE AND SIGNIFICANCE

Rice is an important element of the Mediterranean cuisine. Rice is imported to Lebanon as pre-packed, or unpacked and then, packed inside the country or sold as unpacked. This fact makes the rice supply in Lebanon more prone to contamination with AFs due to high humidity and temperature during transportation and storage. Therefore, the objective of our study is to assess the quality of packed rice marketed in Lebanon in terms of AFB1 and determine the exposure to this toxin from the consumption of rice. For this, the seasonal effect of rice packed in Lebanon, type of rice, presence of a food safety management system certification, time between the production/packing date of rice and the purchasing date from the retailers, country of packing, country of origin and grain size will also be assessed. In parallel, consumption patterns of rice in Lebanon will be determined, using food frequency questionnaires. Then, the liver cancer risk from AFB1 in rice will be calculated. Since the Lebanese Standards Institution (LIBNOR) has implemented a Lebanese standard of 2 μg/kg as a maximum level for AFB1 in rice, our work will be compared to the local and the international MRLs.

2.3 HYPOTHESES

H1: Seasonal effect of rice packed in Lebanon: Warm and humid seasons will enhance the production of AFB1 in rice compared to cold and dry seasons. According to the literature, the conducive production conditions for the aflatoxin biosynthesis gene cluster (~80 kb DNA region) are the optimum temperature (28–37 ºC) and *a*w (> 0.95),

while the non-conducive conditions are at high (> 37 ºC) or low (< 20 ºC) temperature and low *a*w (< 0.93) (Mannaa & Kim, 2017).

H2: Type of rice: Brown rice will tend to be more contaminated with AFB1 than white rice, since upon milling brown rice to white rice, the hull, the germ and the bran layer of the rice along with the molds and AFB1 will be eliminated during this process. According to the literature, brown rice presented higher levels of total AFs compared to white rice (Morrison, Ledoux, Chester, & Samuels, 2019).

H3: Presence of certification: Rice brands with food safety management system certification (ISO 22000, HACCP, FSSC 22000 …) will tend to be less contaminated with AFB1 due to the convenient storage conditions and quality control practices.

H4: Time (number of weeks) between the production/packing date of rice and the purchasing date from the retailers: Rice stored in an unfavorable environment where hotspots are formed and humidity surpasses the equilibrium relative humidity of the grains, retains moisture and presents increased aw levels which leads to fungal growth and AF production (Daou et al., 2021).

H5: Country of packing of rice: Rice packed in developing countries will tend to have higher levels of AFB1 compared to developed countries since in developing countries, good manufacturing and storage conditions may not be implemented.

H6: Country of origin of rice: Rice cultivated in developing countries will tend to have higher levels of AFB1 compared to developed countries since in developing countries, antifungal pesticides use and post-harvest storage conditions tend not to be properly fulfilled.

H7: Grain size of rice: Long grain rice will tend to have higher concentrations of AFB1 than short grain rice due to the larger surface area (Osman, Abdelgadir, Moss, & Bener, 1999; Reiter, Vouk, Böhm, & Razzazi-Fazeli, 2010).

CHAPTER 3

MATERIALS AND METHODS

3.1 SAMPLE COLLECTION

We screened the Lebanese market for white, parboiled and brown rice brands during the month of September 2020. During the first collection in winter, a total of 32 brands of packaged rice were collected from retailers located in Beirut area during the month of February 2021, while a total of 22 brands of packaged rice were collected from retailers located in Jbeil area during the month of March 2021. During the second collection in Spring, a total of 26 brands of packaged rice were collected from retailers located in Beirut area, while a total of 20 brands of packaged rice were collected from retailers located in Jbeil area and a total of 5 brands of packaged rice were collected from a retailer located in Jounieh area during the month of May 2021. Ten brands were not found in the market during the second collection, while seven additional brands were collected.

Screening of AFB1 will be performed using the ELISA technique.

Therefore, the independent variables will include:

- Seasonal effect of rice packed in Lebanon since AFB1 production is influenced by temperature and humidity;
- Type of rice (white vs. parboiled vs. brown) since brown rice tends to have higher AFB1 levels as its white counterpart underwent de-hulling and thus molds and their AFB1 were removed in the process.
- Presence of a food safety management system certification (ISO22000, HACCP, FSSC22000 …), since this implies better storage conditions and quality control measures;
- Time (number of weeks) between the production/packing date of rice and the purchasing date from the retailers which reflects the storage conditions of the rice bags in the food shops since storing in a dry place decreases the risk of its contamination;

- Country of packing of rice which reflects the quality of the post-harvest and storage practices of rice;
- Country of origin of rice which reflects the quality of the agricultural and manufacturing practices of rice;
- Grain size of rice which reflects the capacity of the surface area in retaining AFB1.

The information concerning the production date, type of rice, presence of a FSMS, country of packing, country of origin and grain size, were collected from the packaging information, from the manufacturing industries by phone call and by the industries' website.

3.2 SAMPLE PREPARATION

The rice samples were stored in a cool place at LAU Beirut with no direct contact with light. The first sample preparation of the first rice collection took place at LAU Beirut's lab on the 15th of March 2021, while the second sample preparation of the second rice collection occurred during end of May 2021. A sample was ground and thoroughly mixed before moving to the extraction step. As per the ELISA r-biopharm manual, 5 g of ground rice sample was weighed and placed in a container with an addition of 25 ml of 70 % methanol. Each container was shaken vigorously with a vortex for three minutes and then centrifuged (10 min / 3500 g/ room temperature). Afterwards, 1 ml of the separated solution was diluted with 1 ml of distilled or deionized water. When performing the test, 50 µl of the diluted solution was used per each well. An additional dilution of the sample is needed in case the aflatoxin concentration is expected to be higher.

3.3 SAMPLE ANALYSIS BY ELISA

The first analysis took place during March 2021, while the second during June 2021. For the first and second analysis, 60 and 57 wells were added into the microwell holder for all standards and samples to be used. The standard and sample places were recorded. Then, 50 µl of the standard or prepared sample was pipetted into separate wells while using a new pipette tip for each standard or sample. Afterwards, 50 µl of enzyme conjugate (red cap) was added to the bottom of each well, and 50 µl of anti-

aflatoxin antibody solution (black cap) was also added to each well. Afterwards, the plate was manually shaken in order to mix all the added reagents and then incubated for 30 min (+/- 1) at room temperature (20 - 25 °C). Moreover, the liquid was poured out of the wells into a sink. The microwell holder was tapped upside down three consecutive times on a clean paper towel to take off any existing liquid from the wells. The wells were loaded with 250 µl of washing buffer. Then, they were emptied for another time and evacuated from any present liquid. The washing procedure was performed another two times.

Moreover, 100 µl of substrate/chromogen (brown cap) was then added to each well. The plate containing the mixture was manually shaken and incubated for 15 min (+/- 1) at room temperature (20 - 25 °C) in the dark. Finally, 100 µl of stop solution (yellow cap) was added to each well. The plate containing the mixture was manually shaken, and the absorbance was measured at 450 nm. Reading and quantification were done within 15 minutes of adding a stop solution, by the intermediate of a microtiter plate spectrophotometer. For each collection, the analysis was performed in duplicate.

The estimation of the AFB1 concertation relies on constructing the standard curve which is illustrated based on the absorbance of known concentration of AFB1 standards (0, 1, 5, 10, 20 and 50 µg/kg). Values calculated for the standards are entered in a system of coordinates on semilogarithmic graph paper against AFB1 concentration [µg/kg]. AFB1 concentration in µg/kg corresponding to the absorbance of each sample can be read from the calibration curve (**Figure 3**).

The results were illustrated by the RIDA®SOFT Win (Art. No. Z9999) software that evaluates the RIDASCREEN® enzyme immunoassays.

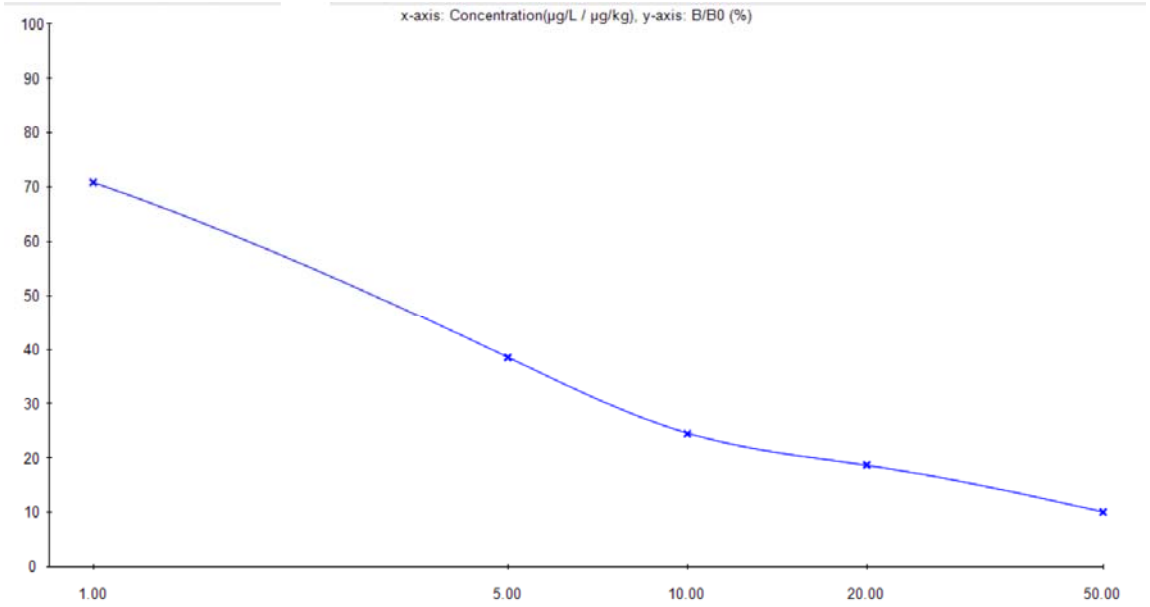


Figure 3 AFB1 Standard curve

3.4 MOISTURE CONTENT ANALYSIS

3.4.1 QUALITY OF RICE AND MOISTURE CONTENT

The quality, including the moisture content of rice, is closely related to fair trade because prices or acceptance criteria are specified based on it. Among the physical quantities determining the quality of rice, moisture content is linked to the stability of rice when stored for a long period of time.

Several practical standardized air-oven methods introduced by different official institutes or societies were used throughout the years in order to determine the moisture content of rice based on drying whole or ground grains over s specific period of time. The "Association of Official Analytical Chemists (AOAC), 1980" has been highlighted as one of those standard procedures (Chen, 2003).

3.4.2 MOISTURE CONTENT ANALYSIS: ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS (AOAC)

The moisture content analysis of the first collection took place during March 2021, while that of the second collection took place during June 2021. The 54 rice samples of the first collection and the 51 rice samples of the second collection were

analyzed in the laboratory at LAU Byblos. The moisture content analysis of each collection was performed once. The samples were previously grounded during the sample preparation for the ELISA analysis at LAU Beirut's laboratory.

In cooled and weighed crucible, 2g of the well mixed sample is weighed and added to it. The weight of each crucible containing the sample is registered. Afterwards, the air-oven is heated and maintained at a temperature of 130±3°. Then, all the crucibles are transferred to the air-oven for 1 hr drying (1 hr drying period begins when oven temperature is actually 130°). When the drying step is completed, the crucibles were taken out of the oven. The weighing occurred as soon the crucibles have reached room temperature. The weight of the crucible with the dried sample is registered (AOAC, 1980).

3.4.3 MOISTURE CONTENT IN RICE CALCULATION

The moisture content in rice is determined on wet basis (wb) as follows using oven drying procedures (IRRI, 2018):

$$MC_{wb} = \frac{Wi - Wf}{Wi} \times 100$$

MC_{wb} = Moisture content wet basis [%]

W_i = Initial weight

W_f = Final weight

According to LIBNOR, the moisture level of the rice grains should be 14% or less of the weight (Lebanese Standards, 2013, p. 3).

3.5 DETERMINATION OF EXPOSURE TO AFB1 FROM RICE CONSUMPTION IN LEBANON

The average consumption of rice in Lebanon (g/day) was assessed using the results from a food frequency questionnaire (FFQ)-based senior study being conducted in parallel by LAU.

Two hundred participants filled the FFQ where approximately 53% of them were females and 47% males. Different governorates were proportionally represented according to the number of households in each of them. The average consumption of dry rice in Lebanon was 68.7 g/day, while the average body weight of participants was 70 kg.

Therefore, the exposure level to AFB1 from rice consumption in Lebanon will be calculated by multiplying the average AFB1 determined from our study by average rice consumption as follows (Panrapee, Phakpoom, Thanapoom, Nampeung, & Warapa, 2016):

Exposure (ng/kg body weight/day) =

$$\frac{Contamination\ level\ (ng/g)\ x\ Amount\ consumed\ (g/day)}{Body\ Weight\ (Kg)}$$

3.6 LIVER CANCER RISK FROM AFB1

According to the Joint FAO/WHO Committee on Food Additives (JECFA), even a very low exposure level to AFB1 (1 ng/kg body weight/day) may increase the incidence of liver cancer. Therefore, it is proposed that, for non-European countries, an ingestion of 1 ng/kg body weight/day of AFB1 will result in an incidence of 0.083 cases of liver cancer per year per 100,000 persons. Therefore, the liver cancer risk based on the overall daily exposure to AFB1 (ng/kg body weight/day) from rice is calculated as follows (JECFA, 1999):

Liver cancer risk from AFB1 =

$$\frac{Exposure\ to\ AFB1\ (ng/kg\ body\ weight/day)\ x\ 0.083\ cancer\ cases/\ 100{,}000\ persons}{1\ (\text{ng/kg body weight/day})}$$

CHAPTER 4

STATISTICAL ANALYSIS

AFB1 concentration was determined as a mean of 2 replicate measures. Data was coded and entered into Excel and then extracted to SPSS V27 for further analysis. Testing for normal distribution of the AF concentration showed a strong positive skew in the data that was caused by 3 large values that deemed to be outliers and were removed for analysis (all 3 values had AFB1 concentration above 1). After removal of the outliers the AFB1 concentration was shown to have a normal distribution and hence was analyzed using parametric techniques. Mean and standard deviations were used to assess central tendency and measure of spread. Difference in means between groups was tested using the independent t test for packing season (Lebanon as country of packing), country of packing, FSMS, grain size, common brands between both collections and ANOVA F test for rice type, country of origin and time between packing and purchasing. When the ANOVA F test showed statistical significance for rice type, post-hoc analysis was carried out using the bonferroni correction for pair-wise comparisons which corrects for the family-wise type I error. All analyzes were carried out at the < 0.05 significant level.

CHAPTER 5

RESULTS

5.1 AFB1 CONTENT IN RICE SAMPLES

AFB1 was detected in 105 out of 105 (100%) of the rice samples tested. Average concentration values of AFB1 for each brand from collection 1 and 2 are presented in **Table 4**. Overall average (± standard deviation) of AFB1 in the 105 rice samples was 0.5 ± 0.3 µg/kg. The level of contamination ranged between 0.06 and 2.08 µg/kg. Only one out of the 105 brands (1%) had an average level above the EU and LIBNOR limits (2 µg/kg).

Table 4 AFB1 content in rice samples from collection 1 and 2

COLLECTION 1 (N= 54)		COLLECTION 2 (N= 51)	
Code	**Average AFB1 ± SD (µg/kg)**	**Code**	**Average AFB1 ± SD (µg/kg)**
1	0.41 ± 0.19	1	0.26 ± 0.16
2	0.52 ± 0.45	2	0.95 ± 0.19
3	0.31 ± 0.1	3	0.46 ± 0.21
4	0.36 ± 0.18	6	0.65 ± 0.28
5	0.36 ± 0.12	7	0.36 ± 0.04
6	0.39 ± 0.08	10	0.82 ± 0.37
7	0.36 ± 0.12	11	0.57 ± 0
8	0.39 ± 0.16	12	0.76 ± 0.11
9	0.51 ± 0.16	13	1.71 ± 0.70
10	0.35 ± 0.09	14	0.21 ± 0.13
11	0.25 ± 0.08	15	0.35 ± 0.19
12	0.33 ± 0.16	N3	0.25 ± 0.21
13	0.63 ± 0.04	17	0.89 ± 0.10
14	0.31 ± 0.11	18	0.56 ± 0.06
15	0.50 ± 0.2	19	0.44 ± 0.13
16	0.42 ± 0.2	21	0.44 ± 0.26
17	0.48 ± 0.17	22	0.33 ± 0.25
18	0.66 ± 0.39	23	0.22 ± 0.24
19	0.31 ± 0.12	24	0.29 ± 0.16
20	0.37 ± 0.05	25	0.50 ± 0.16

21	0.42 ± 0.06	26	0.53 ± 0.33
22	0.53 ± 0.12	27	0.39 ± 0.20
23	0.47 ± 0.13	28	0.40 ± 0.15
24	0.41 ± 0.14	29	0.58 ± 0.47
25	0.47 ± 0.08	30	0.16 ± 0.21
26	0.79 ± 0.39	31	0.06 ± 0.07
27	0.41	32	0.09 ± 0.04
28	0.54 ± 0.06	33	0.22 ± 0.03
29	0.56 ± 0.01	34	0.26 ± 0.19
30	0.64 ± 0.06	36	0.36 ± 0.05
31	0.55 ± 0.14	37	0.15 ± 0.04
32	0.62 ± 0.01	38	0.22 ± 0.09
33	0.85 ± 0.41	39	0.26 ± 0.08
34	0.22 ± 0.29	40	0.29 ± 0.17
35	0.40 ± 0.08	41	0.99 ± 0.55
36	0.68 ± 0.16	42	0.32 ± 0.28
37	0.74 ± 0.05	43	0.46 ± 0.54
38	0.60 ± 0.11	44	0.11 ± 0.01
39	0.72 ± 0.05	45	0.08 ± 0.04
40	0.53 ± 0.04	46	0.24 ± 0.11
41	0.70 ± 0.42	47	0.26 ± 0.08
42	0.16 ± 0.21	48	0.28 ± 0.13
43	0.51 ± 0.13	50	0.55 ± 0.21
44	0.55 ± 0.17	51	0.49 ± 0.21
45	0.68 ± 0.26	53	0.48 ± 0.21
46	0.76 ± 0.13	N1	2.08 ± 0.21
47	0.79 ± 0.20	N2	1.20 ± 0.13
48	0.66 ± 0.03	N4	0.46 ± 0.02
49	0.79 ± 0.45	N5	0.54 ± 0.11
N5	0.22 ± 0.29	N6	0.60 ± 0.23
51	0.71 ± 0.34	N7	0.55 ± 0.08
52	0.92 ± 0.02		
53	0.82 ± 0.18		
54	0.98 0.04		

* All data are presented as mean (± SD). AFB1: Aflatoxin B1

5.2 MOISTURE CONTENT (%) IN RICE SAMPLES

The calculated moisture content (%) in all rice samples from collection 1 and 2 are presented in **Table 5**. All rice samples from collection 1 and 2 had a moisture content (%) < 14%.

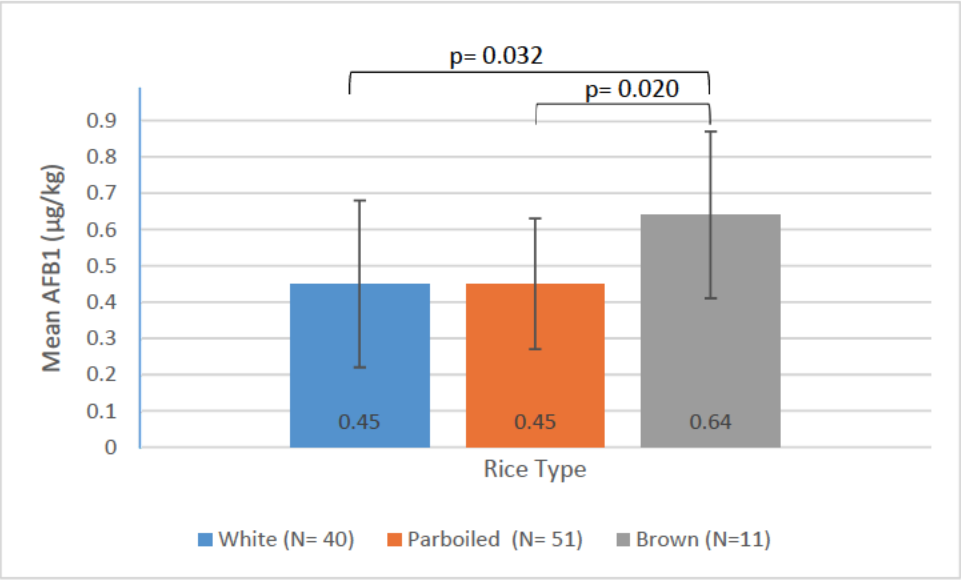
Table 5 Moisture content (%) in rice samples from collection 1 and 2

COLLECTION 1 (N= 54)		COLLECTION 2 (N= 51)	
Code	**Moisture Content (%)**	**Code**	**Moinsture Content (%)**
1	11.78	1	11.27
2	12.49	2	12.19
3	12.06	3	12.09
4	11.96	6	9.60
5	11.79	7	11.99
6	11.16	10	10.95
7	12.56	11	11.59
8	12.34	12	10.84
9	10.81	13	11.24
10	10.60	14	10.64
11	11.70	15	10.61
12	10.05	N3	10.16
13	11.59	17	11.09
14	10.56	18	10.33
15	10.60	19	11.14
16	11.40	21	11.88
17	11.69	22	11.39
18	11.54	23	10.84
19	11.09	24	11.35
20	11.65	25	12.85
21	11.46	26	10.74
22	11.55	27	11.04
23	11.69	28	10.85
24	11.15	29	11.89
25	11.14	30	11.45
26	10.61	31	11.55
27	10.96	32	11.39
28	10.91	33	12.44
29	11.94	34	11.95
30	11.79	36	13.04

31	12.10	37	12.89
32	10.95	38	12.66
33	11.89	39	11.70
34	11.85	40	11.59
35	12.31	41	11.73
36	12.31	42	12.61
37	12.89	43	12.30
38	13.29	44	13.14
39	12.66	45	12.11
40	11.76	46	11.84
41	12.05	47	11.81
42	12.79	48	12.40
43	12.52	50	11.90
44	12.24	51	11.04
45	13.34	53	12.19
46	12.39	N1	11.41
47	13.05	N2	11.36
48	12.15	N4	11.96
49	11.65	N5	10.65
N5	12.16	N6	11.70
51	11.99	N7	11.66
52	11.55		
53	12.20		
54	12.89		

5.3 EFFECT OF RICE TYPE ON AFB1 LEVELS IN RICE

A significant difference was found between white, parboiled and brown rice (p= 0.02). Brown rice had a significantly higher level of AFB1 (0.64 ± 0.23 µg/kg) compared to white (0.45± 0.23 µg/kg) (p= 0.032) and parboiled rice (0.45 ± 0.18 µg/kg) (p= 0.020), while no significant difference was found between white and parboiled rice (p= 0.999) (**Figure 4**).



* All data are presented as N and mean (±SD). Difference in rice type between white, parboiled and brown was tested: ANOVA F, post-hoc analysis using the bonferroni correction for pair-wise comparisons and significance level < 0.05.

Figure 4 Effect of rice type on AFB1 levels in all rice samples

5.4 EFFECT OF DIFFERENT VARIABLES ON AFB1 LEVELS IN RICE

No significant difference was found between rice brands packed in Fall/Winter compared to those packed in Spring/Summer in Lebanon (p= 0.187) (**Table 6**).

No significant difference was found between rice brands packed in Lebanon compared to those packed in other countries (India, Pakistan, Thailand, Italy, France, USA and Spain) (p= 0.093) (**Table 6**).

No significant difference was found between rice from Asian countries (India, Pakistan, Thailand, China), American/ European countries (USA, Italy) and rice with no country of origin information (p= 0.202) (**Table 6**).

No significant difference was found between rice brands with a food safety management system compared to those without a food safety management system of without any related information (p= 0.967) (**Table 6**).

No significant difference was found between long rice grain compared to short/medium rice grain (p= 0.586) (**Table 6**).

No significant difference was found for the time between packing and purchasing of rice bags (p= 0.684) (**Table 6**).

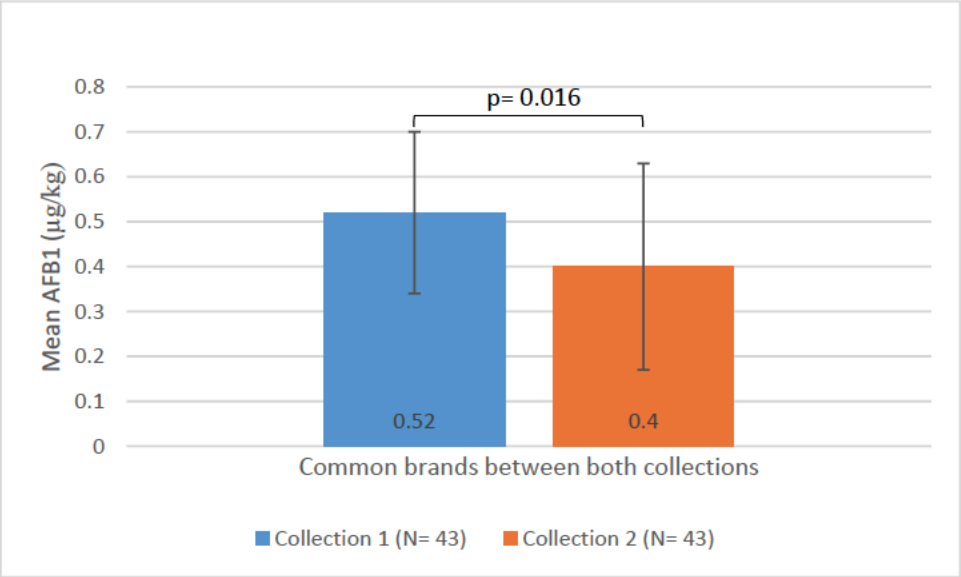
Table 6 Effect of different variables on AFB1 levels in rice samples

Variable	N	Mean	SD	p-
Packing season(Lebanon as country of packing)[a]				
Fall/Winter	45	0.46	0.21	
Spring/Summer	12	0.37	0.21	0.187
Country of packing[a]				
Lebanon	63	0.44	0.21	
Other countries	39	0.52	0.22	0.093
Country of origin[b]				
Developing (India, Pakistan, Thailand, China)	70	0.45	0.19	
Developed (USA, Italy)	24	0.49	0.26	
Not available	8	0.59	0.23	0.202
Food safety management system[a]				
Presence	33	0.47	0.21	
Absence/ Information not available	69	0.47	0.22	0.967
Grain size[a]				
Long	66	0.48	0.19	
Short/ Medium	36	0.45	0.25	0.586
Time between packing and purchasing[b]				
1 to 9 weeks	22	0.48	0.21	
10 to 19 weeks	16	0.43	0.21	
20 to 29 weeks	16	0.41	0.21	
30 weeks and above	28	0.47	0.2	0.684

* All data are presented as N and mean (±SD). Difference in packing season (Lebanon as a country of packing) between Fall/Winter and Spring/Summer, country of packing between Lebanon and other countries, brands with a food safety management system and brands without or with no information related to a food safety management system, and grain size between long and short/ medium rice grain were tested: [a] Independent t Test. Difference in country of origin between Asian countries (India, Pakistan, Thailand, China), American/ European countries (USA, Italy) and rice with no country of origin information, and time between packing and purchasing between 1 to 9 weeks, 10 to 19 weeks, 20 to 29 weeks and 30 weeks and above were tested: [b] ANOVA F.

5.5 COMMON BRANDS BETWEEN BOTH COLLECTIONS AND AFB1 LEVELS IN RICE

The brands from collection 1 had a significantly higher level of AFB1 (0.52 ± 0.18 µg/kg) than the same brands from collection 2 (0.40 ± 0.23 µg/kg) (p= 0.016) (**Figure 5**).



* All data are presented as N and mean (±SD). Difference in common brands between collection 1 and collection 2 was tested: Independent t Test and significance level < 0.05.

Figure 5 Common brands between both collections and AFB1 levels in rice

5.6 EXPOSURE TO AFB1 FROM RICE CONSUMPTION IN LEBANON

The calculated daily exposure to AFB1 ranged between 0.1-2 ng/kg body weight/day with an average of 0.49 ng/kg body weight/day.

5.7 LIVER CANCER RISK BASED ON THE OVERALL DAILY EXPOSURE TO AFB1 FROM RICE IN LEBANON

The liver cancer risk based on the overall daily exposure to AFB1 from rice in Lebanon ranged between 0.005-0.17 cases/100,000 persons/year based on the level of exposure with an average of 0.04 cases/100,000 persons/year.

CHAPTER 6

DISCUSSION

To our knowledge, this is the first study to assess the safety of packed rice marketed in Lebanon, in terms of AFB1 content, and to determine the exposure levels to AFB1 from the rice consumption and the associated liver cancer risk from this toxin. The level of AFB1 in rice samples collected from Lebanon were higher than those reported in other studies. For example, in Turkey, AFB1 concentration had a range between Nd-1.86 μg/kg in rice samples obtained from five provinces of eastern Turkey (Buyukunal et al., 2010). Sun et al. (2011) found that AFB1 was detected in all rice samples collected from China and ranged from 0.1 to 1.4 μg/kg. Al-Zoreky et al. (2019) measured AFB1 in packed basmati, white, parboiled and brown rice sold in Saudi Arabia, and found that AFB1 contamination ranged from 0.014 to 0.123 μg/kg which were within the EU limits. On the other hand, our findings were lower compared to two neighboring countries, UAE and Egypt. As a matter of fact, in UAE, the level of AFB1 contamination ranged between 1.2-16.5 μg/kg in short and long grain rice (Osman, Abdelgadir, Moss, & Bener, 1999). In Egypt, the level of AFB1 contamination ranged between Nd-19.8 μg/kg in rice grains collected from three different districts (Madbouly, Ibrahim, Sehab, & Abdel-Wahhab, 2012). Nonetheless, a previous study conducted by Raad et al. (2014) in Lebanon, measured the dietary exposure to AFB1 from a total diet study in an adult urban population and estimated mean concentration of AFB1 in rice and rice based products, using liquid chromatography. The estimated mean concentration of AFB1 in rice and rice based products was 0-0.010 μg/kg, being the lowest among the food groups. The mean concentration of AFB1 in rice is low compared to LIBNOR and EU limits in both our study and that of Raad et al. (2014). However, the mean concentration of AFB1 in our study was higher compared to Raad et al. (2014). One possible explanation is that Raad et al. (2014) performed the analysis on cooked rice and rice based products which might have underwent processing. In fact, Park et al. (2006) showed that the percentage of AFB1 in contaminated rice decreased by 34% upon ordinary cooking and more than 70 % by pressure cooking. The lower

average concentration of AFB1 in our study could be related to the high barrier protection of the rice packaging bags. Moreover, the moisture levels of the rice grains in our sample were below the maximum level of 14% of the weight, set by LIBNOR which elucidates the low levels of AFB1 in our samples.

Our results showed that, brown rice had a significantly higher level of AFB1 compared to white and parboiled rice ($p=0.02$). These finding are consistent with the literature (Al-Zoreky & Saleh, 2019; Sales & Yoshizawa, 2005; Almeida et al., 2012; Firdous, Ejaz, Aman, & Khan, 2012). For instance, Al-Zoreky et al. (2019) found that AFB1 in white medium and parboiled rice had concentrations < LOD while brown rice had a concentration of 0.014 μg/kg. Sales et al. (2005) demonstrated that AFB1 levels ranged between 0.03–8.33 (Average: 2.6) μg/kg in brown rice and Nd–1.97 (Average: 0.37) μg/kg in polished rice, in Philippines. Nonetheless, when assessing the effect of milling on AF levels, a 78%, 38%, 68% and 82% decrease in mean AF levels were shown from the brown rice to the regular milled rice ($p\leq 0.05$), the regular-milled rice to the well-milled rice ($p\leq 0.05$), the rough rice (before milling) to regular-milled rice (after the first polishing), and to well-milled rice (after the second polishing) ($p\leq 0.05$), respectively. The highest levels of AFs were found in the brown rice, rice hull and the rice bran after the first polishing phase. Another study performed in Brazil showed that among the analyzed rice and its sub-products samples, the average AFB1 levels were 9.09, 6.09, 38.65 and 5.60 μg/kg in rice, rice husk, rice bran and broken rice, respectively (Almeida et al., 2012). It has also been proposed that the high content in fats of the outer layers may promote the attack of molds (Brera, Debegnach, Grossi, & Miraglia, 2004). Moreover, it is important to also consider the initial contamination level of the rice before being exported. On the other hand, when it comes to other grains, Trombete et al. (2014) found that the level of AF was the highest in the bran, followed by whole flour and refined flour. Siwela et al. (2005) demonstrated that AF concentration decreased by 92% when de-hulling maize while Brera et al. (2006) proved that industrial milling reduced four times AF levels in the final product of the processed maize and significantly increased the levels in the germs and bran.

Our study explored the seasonal effect of rice packing in Lebanon on the AFB1 level in rice. Lebanon is characterized by its Mediterranean climate with four different seasons including a rainy period that usually takes place between November and March followed by a dry period which includes very little precipitation (Haddad, Farajalla, Camargo, Lopes, & Vieira, 2014). In general, a hot and dry season in addition to a more humid season lead to higher AF production. In our study, no significant difference was found between rice brands packed in Fall/Winter compared to those packed in Spring/Summer. However, the results were in line with the percentages of moisture content of all rice grains in our sample which were below the maximum level of 14% of the weight. This could be attributed to proper humidity and temperature control in the packing facilities. Our results are in line with Elaridi et al. (2019), where no significant difference was found between fall/winter and spring/summer for the mycotoxins found in baby formulae marketed in Lebanon. Our results are also in agreement with Buyukunal et al. (2010), where no statistical effect of temperature and relative humidity was found on AFB1 manifestation in rice. Nonetheless, it is important to consider that AF contamination increases in fields during drought periods, high temperature with increased CO_2 production and erratic rainfall periods (Akello et al., 2021). Yet in our study, we assessed the seasonal effect of rice packing in Lebanon, located in the Mediterranean region and not rice cultivation in its country of origin where rice is more environmentally exposed to AF contamination. For instance, Panrapee et al. (2015), revealed that rice samples collected during the dry season in Thailand, from December to January, had a lower frequency of AFB1 contamination (10%) than that in samples collected during the rainy season, from June to July. Also, Nguyen et al. (2007) clarified that the rice samples collected in the rainy season had a higher detection ratio and average of AFB1 than the samples collected in the dry season ($p < 0.05$) in Vietnam and concluded that it is crucial to use a convenient method when preserving rice and distributing it to consumers, especially in the markets in order to prevent humidity.

Rice is cultivated in environmental conditions that promote fungal growth and AF contamination. Therefore, the contamination begins within the field (Sales & Yoshizawa, 2005). However, the contamination levels are exacerbated during postharvest sun-drying when the moisture content of the grains remains higher than 14%

(Reddy et al., 2009). Nonetheless, AF contamination of food was not a food safety issue in Europe; however, the current fluctuations in climate patterns have modulated the case (Battilani et al., 2016). In the United States, AF contamination in food is not high in general. Yet, from 2004 to 2013, eighteen reports of food and feed recalls concerning AF contamination were present, even though most of them were associated to dog feed (Mitchell et al., 2016). When assessing the association between the country of origin of rice, whether Asian, American/European or not available and the level of AFB1 in the samples, the results were also not significant. This can be elucidated by the acceptable levels of moisture content found in all the analyzed rice grains in our sample. Nonetheless, 8% of our analyzed samples had no information regarding the country of origin of rice which could also mask the true effect of this independent variable.

This study further demonstrated no significant relationship between rice packed in Lebanon and rice packed in other countries. This could be justified by the acceptable levels of moisture content found in all the analyzed rice grains in our study.

In the present study, there was no statistically significant difference between the presence or absence/ no information of a food safety management system (FSMS) system and the level of AFB1 in rice. It is possible that the significance could not be detected because around 68% of the brands had no information regarding the FSMS. Therefore, in order not to make assumptions, we combined them into the "absence/not known" category. However, the lack of information regarding FSMS does not erase the probability towards the presence of an unillustrated FSMS. On the other hand, it is also highly possible to have industries with FSMS but not abiding by the food safety guidelines or presenting fake certificates. It is also important to acknowledge the difficulty of developing countries and emerging economies in complying with the food safety standards (Trienekens & Zuurbier, 2008). As a matter of fact, Abebe et al. (2020) found that food processors in Lebanon who have executed ISO 22000 (50%), Hazard Analysis and Critical Control Point (HACCP) (40%), and International Organization for Standardization 9001 (ISO 9001) (25.5%), have not executed industry-based more effective FSMSs such as British Retail Consortium (BRC)**,** Safe Quality Food (SQF), Foundation for Food Safety Systems Certification 22000 (FSCC 22000), or International

Featured Standard (IFS). Nonetheless, AFB1 contamination can occur in rice during the cultivation process before being exported to other countries which adds higher requirements on FSMS. Thus, it is crucial to establish an integrated system based on the HACCP approach from field to consumer in order to control AFB1 so it does not exceed the limits set by the legislation. Following good agricultural, storage, manufacturing and distribution practices is important to decrease as much as possible the level of AFB1 before packing rice by reputable industries (Ferre, 2016). Regulation, monitoring and supervision should be adopted, especially in developing countries where they are not endorsed and therefore, lead to food shortage and exacerbate the economy (Daou et al., 2021).

In our sample, long grain rice had a higher concentration of AFB1 than short grain rice. Although our result did not reach significance, it was in line with other studies (Osman, Abdelgadir, Moss, & Bener, 1999; Reiter, Vouk, Böhm, & Razzazi-Fazeli, 2010). When performing the survey of the occurrence of AFB1 in rice consumed in the United Arab Emirates, Osman et al. (1999), found that the mean concentration of AFB1 in sound (1.3 μg/kg ± 1.2), moldy (15.7 μg/kg ± 0.9) and insect-damaged (17.4 μg/kg ± 2.1) long grain rice was higher than sound (1.3 μg/kg ± 0.6), moldy (10.4 μg/kg ± 1.3) and insect-damaged (13.8 μg/kg ± 0.3) short grain rice. Moreover, Reiter et al. (2010) found that, out of 71 analyzed long grain rice samples, 24 were AFB1 positive with a concentration ranging from 0.45 to 9.40 μg/kg, while out of the 5 analyzed short grain rice samples, none was found to be AFB1 positive. According to the LIBNOR standards, long grain rice is characterized by having a length > 6mm with a length/width ratio > 2 but < 3, or, a length > 6mm with a length/width ratio ≥ 3; medium grain rice is characterized by having a length > 5.2 mm but < 6mm with a length/width ratio < 3; while short grain rice is characterized by having a length ≤ 5.2 mm with a length/width ratio < 2 (Lebanese Standards, 2013, p. 7). On the other hand, when it comes to other grains, Akello et al. (2021) found that AF (52%) prevalence was higher in maize compared to small grains (13–25%). Therefore, the higher level of AFB1 in long grain rice in our sample could be due to the higher surface area of this type of rice which might attract more molds and thus AFB1.

When assessing the time between packing and purchasing of rice brands and the level of AFB1 in rice, no significant association was found. Therefore, we speculate that the packaging of rice has good barrier properties which decrease any environmental influence on the quality of rice. In addition, we purchased the rice bags from reputable supermarkets which tend to have good storage practices; however, these bags were not vacuumed in general. As a matter of fact, Bauchet et al. (2020) found that hermetic (airtight) storage bags significantly reduced AF levels in maize after 3 to 4 months of storage and reduced the probability of exceeding the safe to eat limits by 30%. Hermetic bags limit AF contamination by limiting oxygen, increasing carbon dioxide and killing pests on the grains during storage (Ng'ang'a, Mutungi, Imathiu, & Affognon, 2016).

Our results showed a significant difference ($p= 0.016$) between the brands of both collections and the level of AFB1. We speculate that this difference could be due to the inconsistency of the manufacturing practices at the processing and packing sites which increase the susceptibility of AFB1 contamination in rice. Inadequate storage conditions of temperature and humidity, not discarding rice grains with symptoms of fungal contamination can lead to an increased contamination level (Ferre, 2016). As a matter of fact, Tang et al. (2019) demonstrated that the risk of AF accumulation was high in rice samples with high proportions of impurities, and chalky grains. Magan and Aldred (2007) stated that, during post-harvest, interaction between mycotoxigenic fungi and insect pests in stored grain ecosystems enhances the production of mycotoxins. Moreover, when analyzing the presence of AF in the maize supply chain in Congo, Kamika, and Tekere (2016) discovered that AF occurrence rate increased from 32% during pre-harvest to 100% at retail level, proving that the contamination was worsened during the storage, when the maize progressed at the value chain.

For AFs, the tolerable daily intake is not considered a safety factor because the intake of those toxins must remain as low as possible. Accordingly, the PMTDI of 1 ng/kg body weight/day of AF might be considered a guiding value when evaluating the risk of AF from food (WHO 1998). Based on AFB1 levels in our sample, the calculated daily exposure to AFB1 ranged between 0.1-2 ng/kg body weight/day with an average of 0.49 ng/kg body weight/day below the PMTDI. To our knowledge, no study has

assessed the exposure of the Lebanese population to AFB1 from rice consumption. However, the average dietary exposure to AFB1 of an adult urban population estimated by Raad et al. (2014) was 0.63–0.66 ng/kg body weight/day. As a matter of fact, the exposure level to AFB1 calculated in our study was higher than the dietary exposures to AFB1 from rice and wheat consumption in adults (<LOD-0.018 ng/kg body weight/day) and children (<LOD-0.035 ng/kg body weight/day) from France (Sirot, Fremy, & Leblanc, 2013). On the other hand, the exposure level to AFB1 calculated in our study was lower compared to those reported by other authors in other countries. For instance, the exposure level to AFB1 from rice consumption in the Philippines was estimated between 0.1 and 7.5 ng/kg body weight/day (Sales & Yoshizawa, 2005). In Sweden, the exposure level for high rice consumers was 2-3 ng/kg body weight/day (Fredlund et al., 2009). In Iran, it ranged between 1.4-5.8 ng/kg body weight/day for average consumers (Rahmani, Soleimany, Hosseini, & Nateghi, 2011). While in Pakistan, the mean level of exposure to AFB1 from rice and rice products ranged between 22.2-22.3 ng/kg body weight/day (Iqbal, Asi, Hanif, Zuber, & Jinap, 2016).Therefore, even though the results from different studies are useful references, the comparisons should be delicately assessed since the studies may be different in terms of methodology, model used to assess the dietary exposure, the limits of detection/quantification of the analytical technique, the types of rice and rice products included in the studies, the degree of preparation of rice and the consumption patterns that might change between different locations and over time because the contribution of rice to the daily exposure is not only related to level of AFB1 in rice but also to the amount of rice ingested by the population. Therefore, if rice presents a high source of AFB1 in a specific country, it might not be the case for other countries. When it comes to Lebanon, Nasreddine et al. (2019), proved that the consumption of cereals including refined grains such as white rice has increased from 1997 to 2008/-2009. Thus, even though the calculated average daily exposure to AFB1 in our study is not very high, it is highly important to reduce the levels as low as possible in order to prevent the AFB1 toxic effects with higher consumption of rice.

The liver cancer risk based on the overall daily exposure to AFB1 from rice ranged between 0.005-0.17 cases/100,000 persons/year with an average of 0.04 cases/100,000 persons/year. When compared to other countries, the cancer risk in

Thailand was estimated to be 0.011 cases/100,000 persons/year at a mean consumption of brown and color rice (Panrapee, Phakpoom, Thanapoom, Nampeung, & Warapa, 2016). Majeed et al. (2018), found that the mean cancer risk based on polished rice exposure level to AFB1 was 0.070 adults and 0.071 children cases/100,000 persons/year in South Punjab population, and 0.122 adults and 0.127 children cases/100,000 persons/year in North Punjab. Moreover, the cancer risk based on the exposure to AFB1 from rice consumption in Japan was 0.031 cases/100,000 persons/year in children aged between 7–14 years while it was 0.021 cases/100,000 persons/year (Sakuma et al., 2013). Our results are comparable to those of Raad et al. (2014) where they estimated the cancer risk to be 0.0527–0.0545 cases/100,000 persons/year in Lebanon, based on the mean dietary exposure level to AFB1. As a matter of fact, Lebanon (and most of the Middle Eastern countries) is characterized by a higher prevalence of hepatitis B compared to Western Europe (Soubra, Sarkis, Hilan, & Verger, 2009). Precisely, the percentage of HbsAg carriers is estimated at between 5 and 15% of the middle eastern population (Toukan et al., 1990; Qirbi & Hall, 2001), while in Western Europe, it is estimated to be between 0.5 and 2% (Damme, Herck, Leuridan, & Vorsters, 2004). The possibility of having liver cancer due to AF among populations where chronic hepatitis is prevalent is higher compared to populations where it is low (Soubra, Sarkis, Hilan, & Verger, 2009). Moreover, in Lebanon, the incidence of liver carcinoma has been increasing throughout the years. Between 2003 and 2008, the incidence among men and women has increased from 1.8 to 4 cases per 100,000 and from 1.5 to 3.9 cases per 100,000, respectively (Shamseddine et al., 2014). The number of new liver cancer cases in 2020 reached 172 or 1.5% of total cancer cases while the number of liver cancer death reached 168 or 2.6% of total cancer death cases (IARC, 2021). Therefore, even though the liver cancer risk based on the overall daily exposure to AFB1 from rice is not extremely serious, it is crucial to routinely perform surveillance strategies and monitoring programs to ensure minimal AFB1 contamination of rice and halt any possible rise in liver cancer cases.

The strengths of our study include the fact that it was the first of its kind in Lebanon to assess the safety of packed rice marketed in the country, in terms of AFB1 content, and to determine the exposure levels to AFB1 from the rice consumption and the associated

liver cancer risk from AFB1 in Lebanon. In addition, we performed the analysis on packed rice, as the majority of Lebanese population purchase packed rice. Most of our samples were Lebanese brands since due to the current Lebanese pound exchange rate crisis, imported food products became no longer affordable to the majority of Lebanese citizens, and thus there has been a shift towards purchasing local and more affordable brands. Nonetheless, the performed moisture content (%) analysis was in line with our results.

The limitations of our study should be considered. First, there were some outliers in the results. This might be due to the high chance of false positive or negative results caused by insufficient blocking of the surface of microtiter plate immobilized with antigen and in order to address this in future studies, analysis should be done in triplicates. We performed the analysis in duplicates due to budgetary limitations. Moreover, around 50% of the samples had no information regarding the FSMS. We could not reach out to all the food companies and gather straight answers as most of them were not completely cooperative which explains why we combined them into the "absence/not known "category. Also, around 20% of our samples did not have production date information. Thus, this might have masked the real association since we could not assess the packing season effect of those brands on the level of AFB1. Nonetheless, we could not perform our analysis on unpacked rice samples because of mobility restrictions due to the COVID-19 pandemic lockdown and road closures. Furthermore, we could not find an association when assessing the time between packing and purchasing and the level of AFB1, as we purchased the analyzed brands from highly reputable food stores with good storage conditions instead of diversifying them to include smaller food shops. Although ELISA is reliable, HPLC is the golden analytical method when measuring AFB1. Future studies must validate ELISA for determining AFB1 in rice in particular by repeating the work using HPLC.

CHAPTER 7

CONCLUSION

In conclusion, rice is one of the world's most staple food products. AFB1 contamination in rice is highly present around the world. Our study showed that 99% of the tested rice samples were in compliance with EU and LIBNOR limits in terms of AFB1 contamination. Brown rice had higher AFB1 levels than white and parboiled rice while a significant difference was found between both collections for the same brands. Our study suggests that AFB1 contamination of rice marketed in Lebanon is currently not a major public health concern. However, surveillance strategies and monitoring programs must be routinely performed to ensure minimal AFB1 contamination of rice. Nonetheless, it is advised to purchase packed rice brands, with food safety management system certification, and from reputable food markets, in addition to properly store the rice in households, in order to decrease the risk of AFB1 contamination.

Future studies should assess the level of AFB1 in unpacked rice sold in different areas and food markets across Lebanon in order to have a general insight regarding the quality of rice purchased and consumed in Lebanon. Routine monitoring must be carried out to take into account smuggled and emerging brands into the Lebanese market. Moreover, future studies should analyze, in addition to the moisture content of the grains, their a_w, since it is an important indicator as well. Finally, results from ELISA should be validated against the gold standard method, HPLC.

CHAPTER 8

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