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Ochratoxin A in Rice Marketed in Lebanon: Occurrence and Exposure Level

By

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Dedication Page

To my beloved parents

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Ochratoxin A in Rice Marketed in Lebanon: Occurrence and Exposure Level

Abeer Charara

ABSTRACT

Rice is one of the world's most widely consumed foods. *A. Circumdati, A. Nigri*, *P. verrucosum*, and *P. nordicum*can contaminate rice in subtropical and tropical hot and humid climates and secrete mycotoxins, like the toxic and carcinogenic ochratoxin A (OTA). Our research aims to investigate the amounts of OTA in packaged rice sold in Lebanon and the exposure to this toxin from rice consumption. A total of 105 packed white, parboiled, and brown rice bags were collected, with 86 of them coming from 43 different brands. OTA was measured using Enzyme-Linked Immunosorbent Assay (ELISA) technique. In parallel, two hundred participants completed a detailed food frequency questionnaire to determine rice consumption patterns and, as a result, OTA exposure levels from rice intake in Lebanon. OTA was detected in 105 out of 105 (100%) of the rice samples. The average concentration ± standard deviation of OTA was 0.42 ± 0.09 μg/kg. Contamination ranged between 0.02 and 4.98 μg/kg. Moisture content in all rice samples was below the limit (14%). Only 2 rice samples had an OTA level close to the EU limit (5 μg/kg) and were considered as outliers. A significant difference was found between both collections for the same brands ($p<0.001$). Rice type, 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 OTA in rice. Exposure to OTA from rice consumption in Lebanon was calculated as 0.02-4.89 ng/kg body weight/day with an average of 0.41 ng/kg body weight/day. Future studies should assess OTA in unpacked rice, and routine monitoring must be carried out to consider emerging brands in the Lebanese market.

Keywords: Rice, Ochratoxin A, ELISA, Exposure level, Lebanon

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List of Abbreviations

OTA: Ochratoxin A

FAO: Food and Agriculture Organization

RASFF: Rapid Alert System for Food and Feed

EU: European Union

A.:*Aspergillus*

P.*: Penicillium*

a_w**:**Water activity

IARC: International Association for Research on Cancer

MRLs: Maximum Residual Limits

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 ChromatographywithFluorescence Detection

IAC: Immunoaffinity Chromatography

DLLME: Dispersive Liquid-Liquid Microextraction

HPTLC: High-Performance Thin-Layer Chromatography

UHPLC: Ultra-High Performance Liquid Chromatography

LOD: Limit of Detection

LOQ: Limit of Quantification

LIBNOR: Lebanese Standards Institution

FSMS: Food Safety Management System

CHAPTER 1

LITERATURE REVIEW

1.1 MYCOTOXINS

1.1.1 DEFINITION

Mycotoxins are harmful secondary metabolites with insignificant atomic weight, usually less than 1000 Dalton. Different fungi produce mycotoxins such as *Aspergillus, Penicillium, Alternaria, Fusarium,* and *Claviceps* genus(Zhai et al., 2021). While there are over 300 mycotoxins known, six of them are commonly detected in food, causing unpredictable and persistent food safety issues worldwide (Alshannaq& Yu, 2017).These mycotoxins are:

- Aflatoxins (AFs) produced by *Aspergillus*
- Ochratoxin A (OTA) produced bydifferent species of *Aspergillus* and *Penicillium*
- Patulin (PAT) produced by *Aspergillus*, *Penicillium,* and *Byssochlamys*
- Trichothecenes (TCTs) [Type A: HT-2, T2 toxin, and diacetoxyscirpenol (DAS); type B: deoxynivalenol (DON) and nivalenol (NIV); type C &D] produced by *Fusarium*
- Zearalenone (ZEN) produced by *Fusarium*
- Fumonisins B1 and B2 (FBs) produced by *Fusarium*- (Shi et al., 2018).

In the agricultural fields, several plant crops, such as cereals, nuts, forages, fruits, vegetables, and other by-products, pose a high risk of contamination with mycotoxins (Alshannaq& Yu, 2017). In addition, if animals consume contaminated feed, mycotoxins can be detected in animal-derived foods such as meat, eggs, milk, and milk derivatives (Smith et al., 2016). Moreover, contaminatedfood crops with mycotoxins constitutearound 25% of food crops worldwide (FAO, 2013). Thus, the European Union's (EU) Rapid Alert System for Food and Feed (RASFF) has listed mycotoxins in the 2nd place in terms of the total number of risk notifications (RASFF, 2017).

1.1.2 FACTORS LEADING TO MYCOTOXIN PRODUCTION

Mycotoxin contamination may occur before harvest while the yield plant grows or after harvest when food is prepared, packaged, distributed, and stored (Alshannaq& Yu,

2017). Incorrect agricultural and harvesting procedures and incorrect drying, handling, packing, storage, and transport procedures promote the growth of fungi, increasing the likelihood of mycotoxins formation (Marin et al., 2013; Marroquín et al., 2014). Fungi that infect grains are classified into two major categories and a third intermediate group depending on their dominance at various crop growth and harvest phases as influenced by environmental factors:

1. Field fungus, which can colonize ripening grains on standing crops in the field before harvesting. This category contains *Alternaria* and *Fusarium* species. Most field fungal species do not affect crops after harvesting; nevertheless, they can generate mycotoxins before or shortly after harvesting.
2. Storage fungus, which can be present in small numbers before harvesting, infecting grains after harvesting, and increasing quantity during storage. Storage fungus is mainly *Aspergillus* and *Penicillium* species.
3. The intermediate fungal group consists of fungi that grow in storage if the water activity level stays high. This category contains *Cladosporium*, *Fusarium*, and *Trichoderma* species.

Figure 1 shows the different fungal species and their optimum water activity level for growth **(**Mannaa& Kim, 2017). Thus, water activity (aw) between 0.80 and 0.99 and increased temperatures between 25 and 30°C promote fungal growth and, as a result, increase the chances of mycotoxins presence, particularly in staple foods (Ortiz et al., 2013)**.** Besides, climate change scenarios can impact staple commodity security considerably (Martín et al., 2017). Drought, for example, puts plants under massive stress by lowering their natural immunity to diseases and boosting the generation of reactive oxygen species, which are necessary for the production of mycotoxins (Marin et al., 2013).

Knowing that mycotoxins production is unpredictable, this can pose a considerable challenge to food safety. Thus, applying food safety and quality control measures after food processing can decrease the chance of mycotoxin production (Marin et al., 2013). Codex guidelines serve as an international reference for national food supply and food trading, ensuring that the food people purchase meets acceptable standards and quality regardless of the origin of manufacturing (World Health Organization [WHO], 2018). A few practices are in need to avoid any infectious growth. These practices include keeping the products at low temperature when possible and decreasing the water content of plant seeds after harvesting and while storing (FAO, 2013). It is still debatable regarding fungicides since data suggests that

fungicides residues themselves can be a severe issue on consumers' health (Scanes, 2018).Finally, fungi and seeds influence fungal growth by micro-environmental and intrinsic variables, which differ depending on strain specificity. Several epigenetic and environmentally triggered genes have been identified for multiple mycotoxins, highlighting the genome's significance in mycotoxin formation (Marroquín et al., 2014).

Therefore, following food safety and quality control conditions, such as good agricultural practices (GAP) and good manufacturing practices (GMP), is essential to avoid mycotoxin production (Ünüsan, 2019).

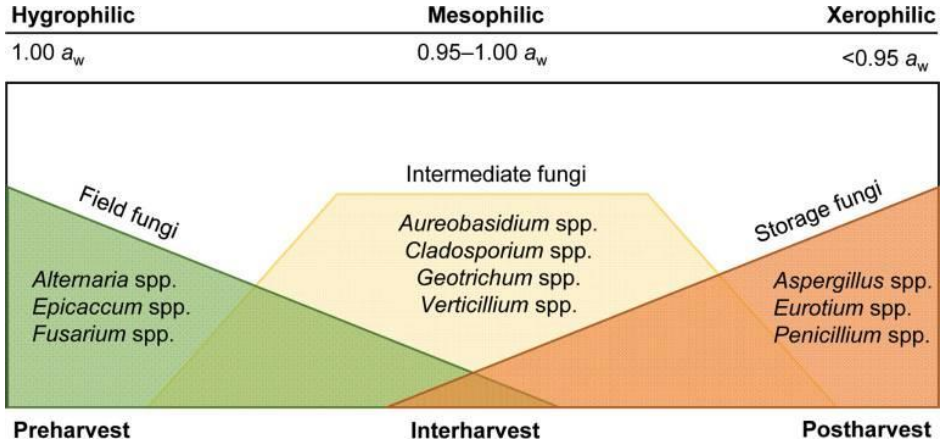

Figure 1. Fungal groups from preharvest to postharvest stages of field crops based on their abundance and dominance. The top scale shows a different classification of fungal species on grains depending on their water activity requirements for fungal growth **(**Mannaa& Kim, 2017)

Mycotoxins pose a risk to human health since they can cause severe and irreversible harm, such as cancer (Omotayo et al., 2019). In the last millennium, the spread of ergotism in Europe resulted in hundreds of thousands of deaths. Between 1942 and 1948, mycotoxins were also the cause of alimentary toxic aleukia, which killed over a hundred thousand Russians. They can cause various illnesses, including headaches and gastrointestinal issues such as abdominal pain, vomiting, and diarrhea. These toxins have been linked to the deaths of millions of people each year (Omotayo et al., 2019).

1.2 OCHRATOXIN A (OTA)

1.2.1 HISTORY OF OCHRATOXIN

In 1965, Ochratoxin A was identified as a metabolite of *Aspergillus ochraceus* after a widescreen of fungal metabolites aimed at finding new mycotoxins. OTA was isolated and chemically analyzed for the first time from a commercial corn sample in South Africa. Later on, it was revealed that contaminating the cornmeal with *Aspergillus ochraceus* was the main reason behind OTA production. After that, *Aspergillus ochraceus* was identified as the first producer of OTA from which it derived its name. Numerous investigations on OTA have been conducted since its discovery, where research has shown that OTA is nephrotoxic, hepatotoxic, embryotoxic, teratogenic, neurotoxic, immunotoxic, genotoxic, and carcinogenic (Malir et al., 2016).

1.2.2 DEFINITION

Ochratoxins, also called phenylalanine–dihydroisocoumarinare compounds, are secondary metabolites generated by a fungus belonging to the *Penicillium* and *Aspergillus* genera. The principal generating species are *Aspergillus Circumdati*, *Aspergillus Nigri*, *Penicillium verrucosum*, and *Penicillium nordicum* (Agriopoulou et al., 2020). There are three types of ochratoxins named ochratoxin A, ochratoxin B, and ochratoxin C., with ochratoxin A (OTA) being the most common and poisonous type where kidney injury is the most sensitive and noticeable outcome. However, the toxin may also affect embryonic growth and the immune system (WHO, 2018). Ochratoxin A ($C_{20}H_{18}C_lNO_6$) has a molecular weight of 403.8. It is a solid crystalline preparation that is white, tasteless, and heat-stable (melting point: 168°C–173°C). Structural analysis of OTA shows that the generation of OTA is by polyketide, which has a dihydrocoumarin moiety linked to l-β-phenylalanine by an amide bond (Figure 2).

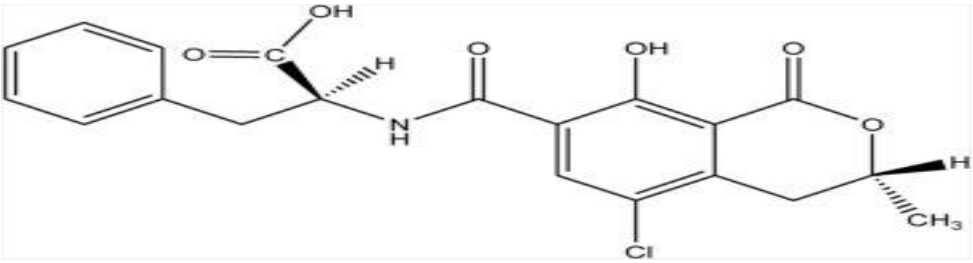

Figure2. Chemical Structure of Ochratoxin A (Zhai et al., 2021)

Moreover and once it enters the bloodstream, OTA has a high affinity to bind to albumin due to its acidic property common in the *Aspergillus* genus. The production of ochratoxin occurs in the aw range of 0.92–0.99 depending on the strain. Ideally, a temperature of 15- 20°C highly produces OTA, and a temperature of 30°C produces less OTA. *Aspergillus* and *Penicillium* grow in a temperature range of 12–37°C and 0–31°C, respectively, meaning that all agricultural regions of the world can produce OTA (Agriopoulou et al., 2020).

1.2.3 OCHRATOXIN METABOLISM & MECHANISM

The gastrointestinal tract, particularly the small intestine, absorbs ochratoxin A efficiently. It is mainly transported to the kidneys via the bloodstream, with smaller quantities seen in the liver, muscle, and fat. The primary metabolite of ochratoxin A in all species studied is ochratoxin alpha, a chlorinated dihydroisocoumarin. Ochratoxin alpha and other minor hydroxylated ochratoxin A metabolites have been less hazardous than ochratoxin A, which has its excretion in urine and feces. Ochratoxin A has a long half-life ranging from 1 - 20 days in animals and up to 35 days in humans (JECFA, 1970).

Moving to the mechanism of OTA in the human body, OTA can induce cell apoptosis and inhibits protein and RNA synthesis. Different studies have found that OTA exposure causes an accumulation of free radicals (Tao et al., 2018). OTA activates the caspase signaling pathway and cell apoptosis by triggering the increase of NADPH and the P450 enzyme. Also, the increase in reactive oxygen species (ROS) generates oxidative stress in the mitochondria and endoplasmic reticulum, which hinder the cell cycle, mRNA splicing, DNA replication, lipid and nucleotide metabolism, and calcium release. All these pathways were known to cause cell death (Tao et al., 2018).

1.2.4 HEALTH CONSEQUENCES

"Mycotoxicosis" is a toxic disease caused by mycotoxins that may be acute or chronic. The inherent toxic properties of the mycotoxin, the quantity and duration of exposure, and the health status of the exposed individual influence their clinical features, target organs, and consequences (Pinheiro et al., 2015). Mycotoxins' ability to adapt to environmental changes indicates that cases of mycotoxin's adverse health consequences will increase in the future (Marroquín et al., 2014). Age, gender, infectious agent exposure, the number of toxins exposed, and the involvement of other mycotoxins (synergistic effects) and pharmacologically active

substances will all affect the impact of mycotoxins on human health and animals (Omotayo et al., 2019). Nevertheless, humans are more affected than animals due to the ability of animals' microbiota to degrade mycotoxins (Ünüsan, 2019).

In the meantime, OTA is a significant public health concern. OTA targets kidneys particularly and is known for its potential to penetrate the placental barrier, exposing a developing embryo to its adverse effects. Also, OTA exposure is known to be a substantial risk factor for both males' and females' reproductive health (Niaz et al., 2020). Besides, OTA contaminations cause different toxicological impacts like teratogenicity, mutagenicity, hepatotoxicity, genotoxicity, immunotoxicity, blood-brain barrier harm, and nephrotoxicity (Kumar et al., 2020).

The OTA toxicity, known as "Ochratoxicosis" can be acute or chronic, where chronic low-dose OTA exposure can be even more dangerous than acute high-dose exposure (Toman et al., 2016). Acute toxicity is rare and depends on OTA doses, species exposed to OTA, and route of administration. It leads to organs bleeding, fibrin thrombi in the spleen, and choroid plexus of the brain, liver, kidney, and heart (Marin et al., 2013).

Chronic ochratoxicosis is of more concern and it is common after prolonged exposure to OTA, leading to different human diseases such as:

- **Balkan Endemic Nephropathy (BEN):** Bilateral chronic renal insufficiency is frequently linked with renal carcinomas. Tubular dysfunction is the most common symptom and it mainly affects the proximal tubule (Marin et al., 2013).
- **Immune system effects:** Malfunction and suppression of humoral and cell-mediated immunity, in addition to increased inflammation due to impaired T or B lymphocyte function and diminished macrophage/neutrophil functions (Marin &Taranu, 2014).
- **Reproductive system effects:** Disruption of the endocrine disruptor and toxicity of reproductive system, with the ability to change the quality and the count of sperm (Malir et al., 2013).
- **Hepatocellular carcinoma (HCC):** Studies have revealed that hepatitis B and C viruses interact in parallel with OTA in the development of HCC (Felizardo, 2013).

The International Agency for Research on Cancer (IARC) classified OTA as a group 2B carcinogen for humans (Bui & Wu, 2015). Furthermore, the European Food Safety Authority (EFSA) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) determined

the latest provisional tolerated daily intakes (PTDIs) for OTA at 120ng/kg BW/week and 100 ng/kg BW/week, respectively (Bui. & Wu., 2015). On the contrary, the limits set by Health Canada and the Scientific Committee of Food (SCF) of the European Union were substantially lower than those calculated by the other organizations, ranging from 1.2 to 5.7 ng/kg BW/day and 5 ng/kg BW/day, respectively (Kuiper et al., 2010).

1.3 RICE

1.3.1 RICE VARIETIES

Rice is the world's most crucial food crop, with more than 90% production in tropical and semi-tropical Asia. According to the Food and Agriculture Organization (FAO), rice is grown in 113 countries and it is the staple food for most of the world's population. Rice provides 27% of dietary energy and 20% of dietary protein in developing countries (FAO, 2004).

Rice is grown in over 40,000 different kinds around the world. However, two kinds are frequently cultivated: *Oryza sativa*, often known as Asian rice, and *O. glaberrima,* African rice. Unlike *O. glaberrima*, which is solely grown in Africa, *O. sativa* is mainly cultivated in Europe, Africa, Asia, Australia, and North and South America.

The Indica, or long-grain rice, and the Japonica, or short round-grain rice, are the main two subspecies of *O. sativa*. The Indica rice species are grown across Asia, whereasthe Japonica rice species are mostly grown and consumed in Australia, China, Taiwan, Korea, the European Union, Japan, Russia, Turkey, and the United States (Ricepedia, 2018). Some of these varieties have fragrant features, and their prices are premiums, such as Thailand's Hom Mali and the various forms of Basmati grown in the Himalayan foothills of India (Haryana and Punjab) and Pakistan (Punjab) (Priya et al., 2019).

The shape or way of processing the grains classifies rice in a variety of ways depending on:

1.3.1.1 SHAPE OF THE GRAINS

It refers to the grain's length and width after cooking:

- **Long grains:** feature a long, slender kernel that is more than four times the width of the grain and they are fluffy and separate after cooking, such as Basmati rice (Chan, 2021).
- **Medium grains:** have shorter and broader kernels and they have soft and semi-sticky texture after cooking, such as Arborio rice (Chan, 2021).
- **Short grains:** have a kernel that is only twice as long as they are wide, and they feature the stickiest texture after cooking, such as Sushi rice (Chan, 2021).

1.3.1.2 PROCESSING AND NUTRITIONAL VALUE OF THE GRAINS

Rice comprises carbohydrates, proteins, and minor amounts of fat, fiber, and moisture (Priya et al., 2019). After harvesting, storing of paddy rice can go from months to years (Al-Zoreky& Saleh, 2019). The hull, an outer protective coat, and the fruit, or rice caryopsis, are the parts that make up the rough rice which is also known as the brown rice. Rough rice goes through dehulling to form brown rice, which comprises the pericarp, seed coat, the germ or embryo, and the endosperm (Priya et al., 2019).Rice processing mostly requires milling, which involves removing the hull and all or part of the bran layer from paddy before converting it into rice. Milling rice aims to remove the husk and bran, resulting in an edible white rice kernel free of contaminants. Some vital elements, such as thiamine and vitamin B, are lost during processing. The milling and polishing procedure eliminates 67% of vitamin B3, 80% of vitamin B1, 90% of vitamin B6, 50% of manganese and phosphorus, 60% of iron, most dietary fiber, and the essential fatty acids found in the primary un-milled type.

Nevertheless, white rice has fewer health benefits than brown rice, where vitamin E, for instance, is found in higher quantities in brown rice (Lee et al., 2019). After milling, the total dietary fiber level of white rice remains lower than that of brown rice (0.7-2% vs. 3-4%, respectively), but the overall caloric content of brown rice is slightly higher due to the fatty content of the bran (Kaur et al., 2016; Fukagawa& Ziska, 2019). On the other hand, white rice is high in minerals like magnesium, manganese, selenium, iron, phosphorus, and vitamins like calcium, thiamin, niacin, folic acid, pantothenic acid, folate, and vitamin E. However, it does not include vitamin C, vitamin A, beta-carotene, lutein, or zeaxanthin (Priya et al., 2019).

1.3.2 SOURCES

Rice is the second most valuable cereal crop in the world, following corn. Nearly 496 million metric tons of milled rice were processed worldwide during the previous harvesting season. Asian countries have long been the leading producers of rice (Shahbandeh, 2021). Rice harvesting occurs at high moisture levels (35-50%) due to its aquatic characteristics. After then, the drying process begins. Rice can become fungus-infested if storage conditions do not meet food safety standards, resulting in the loss of this staple grain and, as a result, a detrimental impact on the economy of rice-producing countries (Škrbić et al., 2017). Each year, 15% of produced rice is wasted primarily on parasite contamination and other potentially harmful species that commonly arise under poor storage conditions. As a result, particularly in Asia, mycotoxin contamination is considered a critical food safety concern (Ruadrew et al., 2013).

According to previous research, *Fusarium*, *Alternaria*, *Penicillium*, *Rhizopus*, and *Aspergillus* are the only fungal genera that can grow in rice and produce secondary metabolites like mycotoxins (Ok et al., 2014). Various parasites produce ochratoxins, but the two most studied and linked species in rice are *Aspergillus ochraceus* and *Penicillium verrucosum* (Ferre, 2016). Furthermore, earlier research has revealed that contamination of cultivated paddy rice (raw, unprocessed rice) with *Aspergillus* spp., *A. niger*, and, in the temperate and colder zones, *Penicillium* species molds, all of which lead to the development of OTAs in harvested paddy rice (Toman et al., 2016). Usually, drying paddy rice before milling is crucial in easing the process and preventing fungi growth. However, when compared to white rice, brown rice has a higher amount of total OTAs. Moreover, compared to raw rough rice, parboiled rice was shown to have higher levels of OTA (Toman et al., 2016; Morrison et al., 2019).

Several studies revealed the contamination of rice with OTAs in Portugal, Spain, Turkey, Egypt, Nigeria, Cote d'Ivoire, Morocco, Tunisia, Jordan, Chile, Vietnam, Japan, Korea, Italy, United States, Iran, Bulgaria, United Kingdom, and Philippines (Bansal et al., 2011; Bui & Wu, 2015).

The European Union (EU) implemented maximum levels of OTA in rice as 5µg/kg (Ferre, 2016). Table 1 illustrates the maximum tolerable limit of OTA in rice and cereals in the EU and other countries

Table 1 Maximum residual limits (MRLs) of ochratoxin A in rice in different countries

Countries/ Organization	MRLs (µg/kg)
Bulgaria	5
Cuba	5
European Union	5
Hungary	5
Iran	5
Ireland	5
Italy	5
Latvia	5
Lithuania	5
Morocco	30
Poland	5
Serbia and Montenegro	10
Singapore	2.5
Sudan	15
Taiwan	5
Turkey	5

1.3.3 PRODUCTION AND CONSUMPTION PATTERNS

In recent years, rice consumption has increased slightly over the world. It increased from 437.2 million metric tons in 2008/2009 to 504.3 million metric tons in 2020/2021 (Shahbandeh, 2021). Asia accounts for more than 90% of global rice production and consumption, with an 87% current share of global rice consumption (Priya et al., 2019). However, rice consumption is not equal across countries; for example, countries with higher urbanization, such as Japan, consume 65 kg per capita, four times less than an overpopulated country like Bangladesh (258 kg per capita) (Milovanovic &Smutka, 2017; Ali, 2019). Figure 3 illustrates the top ten countries in the production and consumption of rice in 2017/2018.

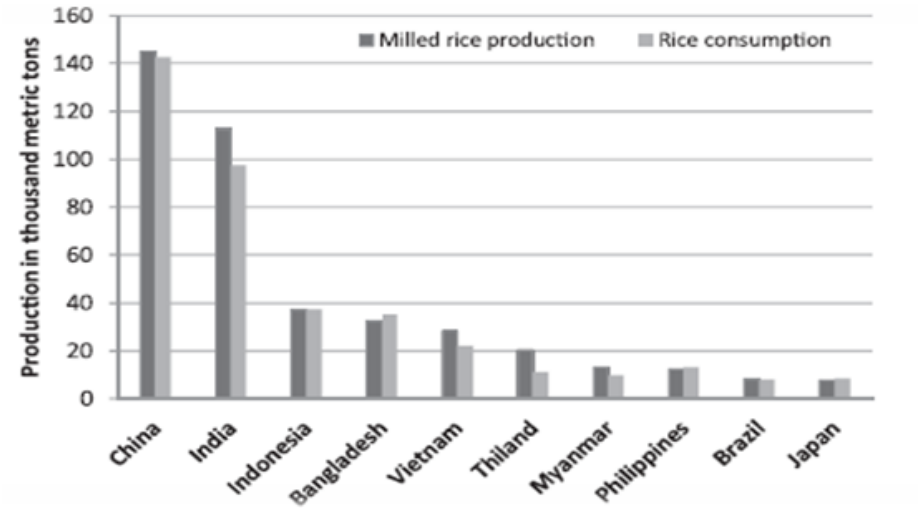

Figure 3. Rice production and consumption in top ten countries in the world in 2017/2018 (Shahbandeh, 2021)

Asia is the continent with the highest rice consumption. China is the first in Asia for rice production and the first in the world for rice cultivation space and yield (Milovanovic &Smutka, 2017) (Figure 3). In 2012, China consumed 67.6 kg of rice per person and harvested 31% of Asia's yield in 2014 (Milovanovic &Smutka, 2017). Furthermore, China ranked first in overall annual rice output in 2015/2016, accounting for 29.2% of global milled rice production (Sun et al., 2017). According to a 2017 study of 154 nations, Bangladesh had the most significant per capita rice consumption with 269 kg, followed by Laos and Cambodia. On the other hand, Serbia ranked at the bottom of the scale with 0.997 kg, Tunisia with 1.22 kg, and Poland with 1.61 kg. Furthermore, according to FAOstat data, the average global rice consumption per capita in 2017 was 79.9 kg, up to 33.4 % higher than ten years before. Rice consumption per capita reached an all-time high of 79.9 kg in 2017 and a low record of 38.8 kg in 1961. Since 1961, the average annual growth rate has been 1.30 %. (Helgi Library, 2021).

Furthermore, Egypt, Turkey, and Iran are the most notable rice-producing countries in the Middle East and North Africa (MENA) region. Between 2011 and 2013, the MENA region consumed an average of 13 million tons of rice, of which 7 million tons were imported (Figure 4) (Nigatu &Motamed, 2015).

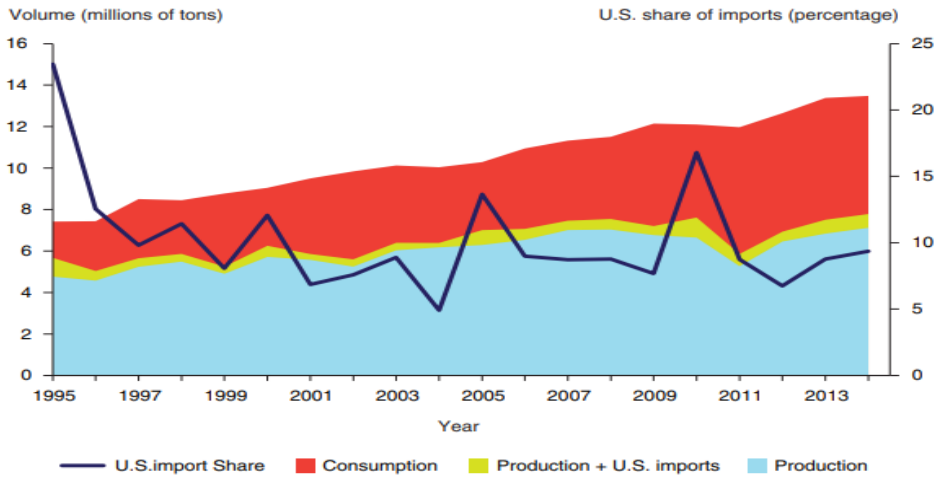

Figure 4 Rice consumption, production, and trade for MENA, and U.S. import share

Egypt has the region's highest rice consumption rate. Regional demand and excess production export capacity, on the other hand, are well addressed. Furthermore, Iran is the second-largest consumer of rice in the Middle East and North Africa (MENA) (Nigatu & Motamed, 2015). Moreover, Iraq and Saudi Arabia are recognized for their significant rice consumption. They import even more rice as a percentage of total consumption than Iran, and imports are expected to rise before 2024. (Table 2) (Nigatu & Motamed, 2015).

	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***

Table 2. Projected MENA consumption and imports for major crop commodities

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.

Lebanon is rated 85th out of 155 nations in terms of rice consumption per capita. In 2017, rice consumption per capita was 14.1 kg, a 13.2% decrease from 2016. In prior years, the highest per capita rice consumption in Lebanon was 16.2 kg in 2016. In 2017, when comparing Lebanon to neighboring countries, rice consumption per capita in Cyprus was 5.09 kg, whereas it was 19.8 kg in Jordan. (Helgi Library, 2021).

1.4 OCHRATOXIN A (OTA) IN RICE

1.4.1 ANALYTICAL METHODS

Many countries have set maximum permissible levels for the presence of OTA in ingested food, necessitating the use of sensitive and selective procedures for determining and quantifying their presence. Thus, OTA is detected using a variety of conventional analytical methods such as high-performance liquid chromatography (HPLC) without or with fluorescence detection (HPLC-FD), liquid chromatography (LC) combined to mass spectrometry (MS) detector, thin-layer chromatography (TLC), and enzyme-linked immunosorbent assay (ELISA) (Ha, 2015):

- **High-Performance Liquid Chromatography (HPLC):** known for its high sensitivity, specificity, accuracy, ease of use, and consistency of results, but it is costly and requires highly qualified people (Burdick, 2019).
- **Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS):** Combining HPLC with MS techniques, LC-MS/MS improves OTA by enhancing trace level identification, selectivity, sensitivity, mass spectral portioning, and the determination of conflicting contaminants. It is, however, quite costly and requires highly skilled people (Iqbal et al., 2014).
- **Thin-Layer Chromatography (TLC):** known for its simple steps, low cost, and durability. Although it is still usable; however, more modern techniques such as HPLC, LC-MS/MS, and ELISA can replace TLC. Its drawbacks include reduced sensitivity and inaccurate results (Iqbal et al., 2014).
- **Enzyme-Linked Immunosorbent Assay (ELISA):** is a labeled immunoassay that is widely considered the gold standard in immunoassays. This susceptible immunological test detects and measures antibodies, antigens, proteins, glycoprotein, and hormones. These products are detected by combining antibodies and antigens to create a quantifiable result. The antigen in the fluid phase becomes immobilized on the microtiter plate in the solid phase. The

antigen then interacts with a specific antibody identified by an enzyme-labeled secondary antibody. The color that appears upon the usage of a chromogenic substrate reflects the antigen's presence. These enzyme-substrate reactions take 30 to 60 minutes to complete and this is after adding a convenient solution. Finally, to detect colored or fluorescent compounds, a microtiter plate reader is used (Sakamoto et al., 2018; Alhajj& Farhana, 2021).

Because of its ease of use, sensitivity, low cost, adaptability, safety, high-throughput minimal sample extraction and sample volume requirement, ELISA is one of the most widely used techniques for OTA analysis. Furthermore, an available, simple, and quick kit produces findings comparable to TLC and HPLC for the quantitative analysis. On the other hand, ELISA necessitates careful supervision for each test to provide correct findings (Iqbal et al., 2014). Nonetheless, due to insufficient blocking of the microtiter plate coated with antigen surface and the possibility of antibody instability, since the antibody is a protein that requires cold transit and storage, ELISA has a high risk of false-positive or negative results (Sakamoto et al., 2018).

1.4.2 OTA LEVELS IN RICE WORLDWIDE

Table 3 summarizes the findings of studies on OTA worldwide.

Table 3. OTA levels in rice worldwide

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; 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; LOD: Limit of Detection; LOQ: Limit of Quantification; Accelerated Solvent Extraction (ASE)

Country	Year of publication	Sample size (rice samples)	Type of rice	Analytical method	Reported OTA level(mean± SDas µg/kg)	Exposure level as ppb or ppm or µg or ng /kg body weight/day	Reference
Korea	2005	88	Polished rice	HPLC-FD	2.1–6.0	-	Park et al., 2005
Portugal	2005	42	Rice	HPLC-FD	0.09- 3.52	-	Pena et al., 2008
Ivory Coast	2006	10	Rice	ELISA	0.16- 0.92	-	Alhajj& Farhana, 2021
Spain	2006	64 non organic & 20 organic	Organic and non organic rice	LC-FD	Organic:1.0 to 7.1 Non organic: 4.3 to 27.3	-	Gonzalez et al., 2006

Vietnam	2007	100	Rice	HPLC-FD	nd-2.78 (0.75)		Nguyen et al., 2007
Tunisia	2008	16	Rice	ELISA	0.8–2.3 (1.4 ± 0.6)	-	Ghali et al.,
Morocco	2008	100	Rice	ASE coupled to HPLC-FD	0.08- 47	0.32	Juan et al., 2008
Turkey	2010	100	Rice	ELISA	0.042 -3.02 (0.81)		Buyukunal et al.
Canada	2011	200	White, brown, red, black, basmati, jasmine and wild rice	IAC-HPLC-FD	Nd-0.49(0.12)	-	Bansal et al.
Nigeria	2011	21		TLC, HPLC	0-341.3 (141.7 ± 25.4)	-	Makun et al., 2011

Brazil	2012	230	rice with the pro- cessing fractions (bran, rice husk and broken)	IAC, HPLC-FD	0.20-0.24	-	Almedia et al., 2012
Ecuador	2013	121 (paddy rice) and 125 (pol- ished rice)	Paddy and polished rice	IAC, UHPLC	Nd	-	Ortiz et al., 2013
Iran	2013	65	Domestic rice	LC-MS/MS	0.65– 11.54 (5.02)	-	Nazari et al., 2013
Pakistan	2013	68	Brown rice	HPLC-FD	12.94 ± 1.98	-	Majeed et al., 2013
South China	2014	370	Rice	DLLME coupled to HPLC	0.30-3.2 (0.85 ±1.2)	-	Lai et al., 2014
China	2014	370	Rice	DLLME Coupled to HPLC- FD.	0.30-3.2 (0.85 ±1.2)	-	Lai et al., 2015

Bohemia	2016	60	White & parboiled rice	HPLC-FD	0.05- 0.17	-	Toman et al., 2016

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 OTA, to determine the exposure levels from the rice consumption and to shed light on the importance of incorporating Maximum Residual Limits for OTA in the related norms by the Lebanese Standards Institution (LIBNOR).

2.2 RESEARCH OBJECTIVE AND SIGNIFICANCE

Rice is imported into Lebanon either pre-packaged or unpackaged and then repackaged or sold unpackaged. Because of the high humidity and warmth in Lebanon's rice supply during shipping and storage, it is more susceptible to OTA contamination.

Our research aims to identify the quality of packed rice sold in Lebanon in terms of OTA and the exposure to this toxin through rice consumption. For this, the packing season, kind of rice, presence of a food safety certification at the rice facility, duration between packing date and purchase date from retailers, region of packing, country of origin, and the grain size were considered. At the same time, food frequency questionnaires to investigate rice consumption patterns throughout Lebanon were used.

Finally, our work will shed light on the importance of incorporating MRLs for OTA in the Lebanese Standards Institution (LIBNOR) since the latter has not yet implemented a Lebanese standard maximum level for OTA in rice.

2.3 HYPOTHESES

H1*:* Seasonal effect: In comparison to cold and dry seasons, warm and humid seasons can boost OTA production in rice and this is because of their ability to develop OTA over a broad temperature range (Esteban et al., 2004).

H2: Type of rice: Brown rice is more likely to be contaminated with OTA than white rice because the hull, germ, and bran layers of the rice, as well as molds and OTA, are removed during the milling process (Morrison et al., 2019).

H3: Presence of certification: Due to convenient storage conditions and quality control measures, rice brands packed by certified with a food safety management system (ISO22000, HACCP, FSSC22000) are less likely to be contaminated with OTA.

H4: The time (in weeks) between the rice's production/packing date and the retailer's purchase date: Rice maintained in an unfavorable environment, where the development of hotspots occurs, and relative humidity exceeds the grains' equilibrium relative humidity, retains moisture, and creates higher aw levels, resulting in fungal growth and OTA generation (Daou et al., 2021).

H5: Country of packing of rice: Rice packed in underdeveloped countries is more likely tohave higher levels of OTA than rice packed in developed countries because of inadequate production and storage procedures in these countries.

***H*6:** Country of origin of rice: Rice produced in developing countries will have higher levels of OTA than rice grown in industrialized countries because of the absence of antifungal pesticides and suitable post-harvest storage conditions in these countries.

***H*7:** Grain size: Long grain rice will have higher OTA concentrations than short-grain rice due to its larger surface area (Osman et al., 1999; Reiter et al., 2010).

CHAPTER 3

MATERIALS AND METHODS

3.1 SAMPLE COLLECTION

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

Screening of OTA will be through using the ELISA technique.

The independent variables will be:

- Packing season of rice because temperature and humidity impact OTA production
- Rice variety (white vs. parboiled vs. brown) because brown rice goes through de-hulling that eliminates molds and associated OTA
- Certification of a food safety management system (ISO22000, HACCP, FSSC22000) indicates improved storage conditions and quality control systems
- Time (in weeks) between the rice production/packaging date and purchase from retailers' date reflects the storage conditions of rice bags in grocery stores since storing in a dry environment minimizes the risk of contamination
- Country of packaging of rice represents the quality of rice storage and post-harvest practices
- Country of origin of rice indicates the quality of the rice's agricultural and manufacturing processes
- Grain size of the rice where it determines the surface area's ability to store OTA

3.2 SAMPLE PREPARATION

The rice samples were kept at LAU Beirut in a freezer (-18ºC). Sample preparations took place at LAU Beirut's laboratories. According to the ELISA kit manufacturer instructions (RIDASCREEN Ochratoxin A 30/15R1312, r-biopharm, Germany), 50ml of ECO extractor was diluted in 450 ml of distilled water (1:10 dilution) for the extraction process. After that, 7g (6.95-7.04 g) of ground rice, that we already grinded, were mixed with 35 ml ECO in 50 ml centrifuge tubes. Each container was shaken briefly by hand and shaken by a vortex for five minutes before centrifugation (5 minutes / 3500 g/ room temperature). Then, buffer salt sachet was dissolved in 1 L of distilled water and shaken well to dissolve. After that, we added 1 ml of wash buffer solution to new tubes with 1 ml of the supernatant using different pipette tips for each tube. Finally, we added 50 µl of the diluted solution to each well.

3.3 SAMPLE ANALYSIS BY ELISA

Wells were added to the microwell holder for all standards and samples to be assessed. Locations of standards and samples were recorded. Then, 50 µl of the standard or prepared sample was pipetted into separate wells using a different pipette tip for each standard or sample. Afterward, 50 µl of enzyme conjugate was added to the bottom of each well and the plate was gently shaken to mix the reagents and was then incubated for 30 minutes (+/- 1) at room temperature (20 - 25°C) in a dark place. After that, liquid was poured out of the wells and tapped the microwell holder three times upside down against absorbent paper to ensure the complete removal of liquid from the wells. Then, 250 µl of washing buffer was added to the wells and emptied for a second time to remove any remaining liquid. Washing procedure was repeated twice.

In addition, 100 µl of substrate/chromogen was added to each well. Then, plate was manually shaken and incubated it for 15 min (+/- 1) at room temperature (20 - 25 °C) in the dark. Finally, each well was loaded with 100 µl of stop solution (yellow cap) to stop any reaction. Absorbance was measured at 450 nm within 15 minutes of adding the stop solution by a plate spectrophotometer.

OTA concentration estimation relies on constructing the standard curve, which is demonstrated based on the absorbance of known concentration of 6 OTA standards (0, 0.03, 0.1, 0.3, 1, and 3 µg/L). Values calculated for the standards are entered in a system of coordinates on semilogarithmic graph paper against OTA concentration [µg/kg]. Through the calibration curve, the OTA concentration in µg/kg corresponding to the absorbance of each sample can be read.

The results were illustrated by the RIDASOFT® Win (Art. No. Z9996) software that evaluates the RIDASCREEN® enzyme immunoassays.

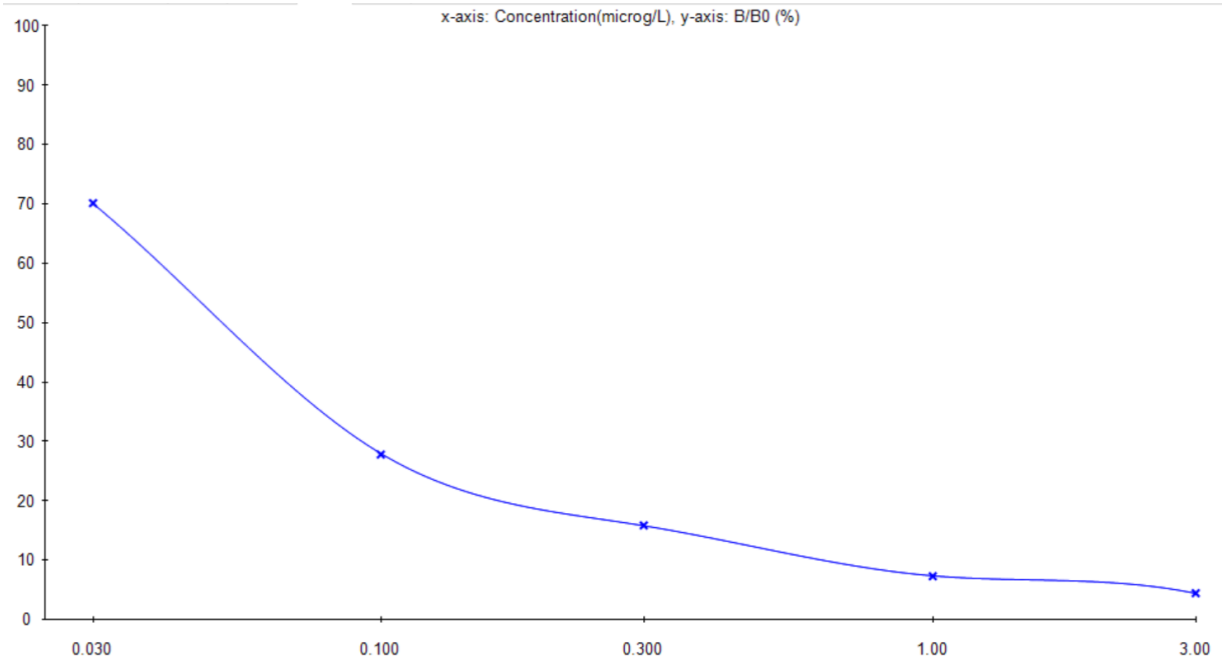

Figure 5.OTA Standard curve

3.4 MOISTURE CONTENT ANALYSIS

3.4.1 QUALITY OF RICE AND MOISTURE CONTENT

The quality, including the moisture content of rice, is directly related to poor trade since prices or acceptance criteria are specified based on it. Consumers among different regions, countries, cities, and urbanization levels tend to perceive rice quality differently. In Southeast Asia,

premium quality is defined by nutritional value, softness, and aroma, while in South Asia, the quality is defined by the physical features of the grains, satiety, and aroma. Moisture content is one of the physical variables that influence rice quality after it has been kept for an extended period (Custodio et al., 2019)

Several practical standardized air-oven methods created by various official institutes or societies were used throughout the years to assess the moisture content of rice based on drying whole or ground grains for a certain period. One of these standard methods has been identified as the "Association of Official Analytical Chemists (AOAC), 1980" (Chen, 2003).

3.4.2 MOISTURE CONTENT ANALYSIS: ASSOCIATION OF AOAC

The moisture content analysis of the first collection took place during March 2021, while that of the second collection was analyzed in June 2021. The first collection's 54 rice samples and the second collection's 51 rice samples were examined at the laboratory at LAU Byblos. Each collection's moisture content was analyzed only once. The samples were previously ground at LAU Beirut's laboratory as part of the sample preparation for the ELISA analysis. In cooled and weighed crucible, 2g of the well mixed sample was weighed and added to it. The weight of each crucible containing the sample was registered. Following that, the air oven was heated and kept at a temperature of 130±3°C. The crucibles were then moved to the air oven for 1 hour of drying. When the drying process was finished, the crucibles were removed from the oven. The weighing began as soon as the crucibles reached room temperature. The weight of the crucible containing the dried sample was reported (AOAC, 1980).

3.4.3 MOISTURE CONTENT IN RICE CALCULATION

The moisture content of rice is assessed on a wet basis (wb) utilizing oven drying methods (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 OTA From Rice Consumption In Lebanon

The average consumption of rice in Lebanon (g/day) was determined using a food frequency questionnaire (FFQ)-based senior study conducted concurrently by LAU.

The FFQ had 200 participants, with nearly 53 % females and 47% males. According to the number of households in each governorate, different governorates were proportionally represented. The average daily consumption of dry rice in Lebanon was 68.7 g, while the participants' average body weight was 70 kg.

As OTA contamination in rice is constant, the amount of OTA exposure from rice intake in Lebanon will be estimated by multiplying the average OTA determined in our study by the average rice consumption, as shown below (Panrapee et al., 2016):

Exposure (ng/kg body weight/day) =

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

3.6 Statistical Analysis

OTA concentration was determined as a mean of 2 replicate measures. Data were coded and entered into Excel and then extracted to SPSS V27 for further analysis. Testing for normal distribution of the OTA concentration showed a strong positive skew in the data that was caused by two large values that were deemed to be outliers and were removed from analysis (the two values had OTA concentration above 1). After removing the outliers, the OTA concentration was shown to have a normal distribution and hence was analyzed using parametric techniques. Mean and standard deviation were used to assess central tendency and measure of spread. The difference in means between groups was tested using the independent t-test for two groups and the one-way ANOVA for more than two groups. When ANOVA test showed statistical significance, posthoc analysis was carried out using the Bonferroni correction for pair-wise

comparisons, which corrects the family-wise type I error. All analyses were carried out at the <0.05 significant level.

CHAPTER 4

RESULTS

4.1 OTA CONTENT IN RICE SAMPLES

OTA was detected in 105 out of 105 (100%) of the rice samples tested. Average concentration values of OTA from collection 1 and 2 are presented in Table 4 and Table 5. Overall average (±standard deviation) of OTA in the 105 rice samples was 0.42 ± 0.09 µg/kg. The level of contamination ranged between 0.02 and 4.98µg/kg. Only two out of the 105 brands had an average level close to the EU limits (5µg/kg) and were removed from the statistical analysis.

Table 4. Minimum, Maximum, & average mean (µg/kg) of ochratoxin A in collection 1 (N=54), collection 2 (N=51) and all samples combined (N=105)

	Minimum (µg/kg)	**Maximum (µg/kg)**	**Average Mean (µg/kg)**
Collection 1 (N=54)	0.22	4.98	0.65
Collection 2 (N=51)	0.02	0.81	0.20
All samples combined (N=105)	0.02	4.98	0.42

4.2 MOISTURE CONTENT (%) IN RICE SAMPLES

Table 5 presents the calculated moisture content (%) in all rice samples from collections 1 and 2. The moisture content (%) of all rice samples from collections 1 and 2 was less than 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**	**Moisture 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		

4.3 EFFECT OF RICE TYPE ON OTA LEVELS IN RICE

No significant difference was found between white, parboiled, and brown rice (p=0.258) (**Table 6**).

Table 6. Effect of rice type on OTA

Rice Type	N= 103	Mean	SD	P-value
White rice	37	0.33	0.38	0.258
Parboiled rice	53	0.51	0.71	
Brown rice	13	0.31	0.18	

* All data are presented as N and mean (±SD). Difference in rice type between white, parboiled and brown was tested using one-way ANOVA.

4.4 SEASONAL EFFECT OF RICE PACKING IN LEBANON ON OTA LEVELS IN RICE

No significant difference was found between rice brands packaged in Fall/Winter compared to those packed in Spring/Summer in Lebanon (p= 0.286) (**Table 7**).

Table 7. Seasonal effect of rice packing in Lebanon on OTA

Packing season (Lebanon as country of packing)	N= 103	Mean	SD	P-value
Fall/Winter	61	0.47	0.69	0.286
Spring/Summer	42	0.35	0.32	

* All data are presented as N and mean (±SD). Difference in packing season (Lebanon as country of packing) between Fall/Winter and Spring/Summer was tested using Independent t-Test.

4.5 EFFECT OF COUNTRY OF PACKING ON OTA LEVELS IN RICE

No significant difference was found between rice brands packed in Lebanon compared to those packed in other countries (Thailand, India, USA, Pakistan, Italy, Spain, and France)(P= 0.989) (Table 8).

Table 8. Effect of rice packing country on OTA

Country of packing	N= 103	Mean	SD	P-value
Lebanon	63	0.42	0.68	0.989
Other countries(India, Pakistan, Thailand, Italy, France, USA and Spain)	40	0.42	0.33	

* All data are presented as N and mean (±SD). Difference in country of packing between Lebanon and other countries were tested using Independent t-Test.

4.6 EFFECT OF COUNTRY OF ORIGIN ON OTA LEVELS IN RICE

No significant difference was found between rice from Developing countries (China, India, Pakistan, Thailand), Developed countries (USA, Italy) and rice with no country of origin information (P= 0.332) (**Table 9**).

Table 9. Effect of country of origin on OTA

Country of origin	N= 103	Mean	SD	P-value
Developing(China, India, Pakistan, Thailand)	71	0.47	0.65	0.332
Developed (USA, Italy)	22	0.31	0.36	
Not available	10	0.26	0.16	

* All data are presented as N and mean (±SD). Difference in country of origin between Developing countries (India, Pakistan, Thailand, and China), Developed countries (USA, Italy) and rice with no country of origin information was tested using one-way ANOVA.

4.7 EFFECT OF A FOOD SAFETY MANAGEMENT SYSTEM ON OTA LEVELS IN RICE

There was no significant difference between rice brands that had a food safety management system and those that did not have one or did not have/present any related information. (p=0.621) (Table 10).

Table 10. Effect of a food safety management system on OTA

Food safety management system	N= 103	Mean	SD	P-value
Presence	33	0.38	0.31	0.621
Absence/ Information not available	70	0.44	0.66	

* All data are presented as N and mean (±SD). Difference between brands with a food safety management system and brands without or with no information related to a food safety management system was tested using independent t-Test.

4.8 EFFECT OF GRAIN SIZE ON OTA LEVELS IN RICE

No significance difference was found between long rice grain and short/medium rice grain (p=0.086). (Table 11).

Table 11. Effect of grain size on OTA

Grain size	N= 103	Mean	SD	P-value
Long	67	0.49	0.65	**0.086**
Short/ Medium	36	0.23	0.33	

* All data are presented as N and mean (±SD). Difference in grain size between long and short/ medium rice grains was tested using Independent t-Test.

4.9 EFFECT OF TIME BETWEEN PACKING AND PURCHASING ON OTA LEVELS IN RICE

No significant difference was found for the time between packing and purchasing of rice bags (p=0.157) (Table 12).

Table 12. Effect of time between packing and purchasing of rice bags on OTA

Time between packing and purchasing	N= 82	Mean	SD	P-value
1 to 9 weeks	21	0.67	0.107	0.157
10 to 19 weeks	18	0.24	0.28	
20 to 29 weeks	16	0.37	0.38	
30 weeks and above	27	0.42	0.27	

* All data are presented as N and mean (±SD). Difference in time between packing and purchasing between 1 to 9 weeks, 10 to 19 weeks, 20 to 29 weeks and 30 weeks and above was tested using one-way ANOVA.

4.10 EFFECT OF COLLECTION ON OTA LEVELS IN RICE

A significant difference was found between both collections of rice bags (p<.001) **(Table 13).**

Table 13. Effect of collection on OTA

Collection	N= 82	Mean	SD	P-value
First	50	0.66	0.72	<.001
Second	53	0.19	0.21	

* All data are presented as N and mean (±SD). Difference between first and second collections was tested using independent t-test.

4.11 EXPOSURE TO OTA FROM RICE CONSUMPTION IN LEBANON

The calculated daily exposure to OTA ranged between 0.02-4.89 ng/kg body weight/day with an average of 0.41 ng/kg body weight/day.

CHAPTER 5

DISCUSSION

Until today, to our knowledge, this is the first study to assess the safety of packaged rice sold in Lebanon in terms of OTA concentration, as well as to quantify levels of OTA exposure from rice intake, and to highlight the necessity of OTA MRLs in LIBNOR. OTA level in rice samples collected in Lebanon (0.42 ± 0.09 μg/kg) was higher than in some studies but lower than others. In Brazil, for example, in the period 2007–2009, OTA concentrations in processed rice and its sub-products (bran, rice husk, and broken) obtained from various regions of the country ranged from 0.20-0.24 μg/kg (Almedia et al., 2012). OTA was not found in any of the rice samples collected in Ecuador, according to Ortiz et al. (2013). In Portugal, Pena et al. (2005) measured OTA contamination in various types of rice from multiple brands, including white rice (long-grain rice, medium-grain rice, raw and pre-boiled), basmati rice, thai rice, wild rice, aromatic rice, and raw brown rice, and noticed that OTA contamination ranged from 0.09 to 3.52 μg/kg, which was within the EU limits (5 μg/kg). In comparison to Morocco and Iran, however, our findings were lower. In fact, in Morocco, the level of OTA contamination in rice ranged from 0.08 to 47 μg/kg (Juan et al., 2008). In rice samples selected at random from local markets in Tehran, Iran, the degree of OTA contamination ranged from 0.65 to 11.54μg/kg (Nazariet al., 2014). Trung et al. (2001), Gonzalez et al. (2006), and Scudamore et al. (1997) reported high amounts of OTA in earlier studies, with levels ranging from 21.3-26.2 μg/kg, 4.3-27.3 μg/kg, and 1.0-19.0 μg/kg, respectively. Nonetheless, Raad et al. (2014) used liquid chromatography to determine the dietary exposure to OTA from a whole diet study in an adult urban population and estimate the mean concentration of OTA in rice and rice-based products. Rice and rice-based items had an estimated mean OTA concentration of 0.680 μg/kg, which was lower than concentrations observed in Biscuits/croissants and Alcoholic drinks (2.844 μg/kg and 1.472 μg/kg, respectively), but higher than bread and almonds (0.298 μg/kg and 0.078 μg/kg, respectively). Our investigation and that of Raad et al. (2014) found that the mean concentration of OTA in rice is low compared to EU limits (5μg/kg). Furthermore, when compared to Raad et al. (2014), the mean concentration of OTA in our sample was lower. One probable explanation is that Raad et al. (2014) examined rice products rather than raw rice alone. Their study was carried out in 2014, where the rice brands available in the Lebanese

market were different from the ones we collected. In fact, Lebanon is currently witnessing an unprecedented economic and political crisis, resulting in emergence of new rice brands, imported and locally packed. Last but not least, the moisture levels of the rice grains in our samples were below the LIBNOR maximum of 14% of weight, which can explain the relatively low levels of OTA in our samples compared to other studies.

Our findings revealed that parboiled rice had a higher level of OTA (0.508 μg/kg) than white and brown rice, and white rice had slightly higher levels of OTA than brown rice (0.326 and 0.313 μg/kg, respectively); however, these differences were not significant (p= 0.258), and these findings are consistent with some studies but inconsistent with others.For instance, Toman et al. (2016) showed that parboiled rice had greater OTA levels than rough raw rice, and Iqbal et al. (2016) observed that white rice had a higher OTA level than brown rice. In contrast, a Brazilian study found that the average OTA levels in rice, rice husk, rice bran, and broken rice were 1.78, 3.91, 11.63, and 1.02 μg/kg, respectively, in the studied rice and its sub-products samples (Almeida et al., 2012).

Our study explored the seasonal influence of rice packing in Lebanon on the OTA level in rice. Lebanon has a Mediterranean climate with distinct seasons: a rainy season from November to March, followed by a dry season with little precipitation (Haddad et al., 2014). In general, increased OTA production is associated with a hot and dry season and a more humid season. Our research showed no noticeable difference between rice brands packed in the Fall/Winter and those in the Spring/summer. The results, however, were consistent with moisture content percentages of all rice grains in our sample, which were below the maximum level of 14% of the weight. These findings could be attributable to the packing facilities' adequate humidity and temperature management. Our findings are consistent with Elaridi et al. (2019), who observed no significant differences in OTA levels in baby formulas sold in Lebanon collected inthe fall/winter and spring/summer seasons. Despite this, because of the wide temperature range of *Aspergillus* and *Penicillium* species, OTA production can occur at any time of year (Agriopoulou et al., 2020). Nevertheless, in our research, we looked at the seasonal effect of rice packing in Lebanon, a Mediterranean country, rather than rice production in the area of origin, where rice is more environmentally prone to ochratoxin contamination. For instance, Nguyen et al. (2007) found that rice samples taken during the dry season in Vietnam had a more detection ratio and OTA average than samples collected during the rainy season.

Rice is grown in a setting that encourages fungus growth and OTA contamination. As a result, contamination begins in the field (Sales &Yoshizawa, 2005). When the moisture content of the grains remains higher than 14% after postharvest sun-drying, this will aggravate the contamination levels (Reddy et al., 2009). Nonetheless, mycotoxin contamination of food was not a food safety concern in Europe; now, current climate trends have changed the situation (Battilani et al., 2016).

When examining the association between the country of origin of rice, whether developing (India, Pakistan, Thailand, China), developed (USA, Italy) or not available, and the level of OTA in the samples, the results were also not significant. These results can be interpreted by the acceptable levels of moisture content found in all the analyzed rice grains in our sample. Nonetheless, 9.7% of the samples we examined lacked information on the nation of origin of the rice, potentially masking the actual influence of this independent variable. Nonetheless, the mycotoxin problem is often more problematic in developing countries in Asia, where climatic conditions and agricultural and storage techniques are favorable to fungus growth and toxin generation (Ali, 2017).Furthermore, according to this study, no significant association was found between rice packed in Lebanon and rice packed in other countries. These results are supported by the permissible moisture content values found in all rice grains examined in our study.

There was no statistically significant difference between the presence or absence/no information of a food safety management system (FSMS) and OTA level in rice in our study. The significance could not be detected because around 68% of the brands lacked information regarding the FSMS. As a result, to avoid making assumptions, we grouped them in the "absence/information not available" category. However, the lack of data on FSMS does not eliminate the possibility of an unillustrated FSMS. In contrast, it is common to have industries with FSMS but not adhere to food safety requirements or present false certificates. It is also essential to identify the challenges that developing countries and emerging economies face in achieving food safety regulations (Trienekens & Zuurbier, 2008). Abebe et al. (2020) discovered that food processors in Lebanon who have implemented ISO 22000 (50%), HACCP (40%), and ISO 9001 (25.5%) had not adopted industry-based FSMSs. However, OTA contamination can occur in rice throughout the growing process before being shipped to other countries, necessitating more stringent FSMS requirements. Thus, from field to consumer, an

integrated system based on the Hazard Analysis and Critical Control Point (HACCP) approach is critical for controlling OTA and ensuring that it does not exceed the legislative limits. Following appropriate agricultural, storage, manufacturing, and distribution processes is essential to decrease as much as possible the level of OTA before packing rice by recognized industries (Ferre, 2016).

In our study, long grain rice exhibited a greater OTA concentration than short grain rice. Although our findings were not significant, long grain rice had a greater OTA concentration in our sample than short grain rice. According to LIBNOR standards, long grain rice is defined as 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 defined as having a length > 5.2 mm but < 6mm with a length/width ratio < 3; and short-grain rice is defined as having a length ≤ 5.2 mm with a length/width ratio <2 (Standards in Lebanon, 2013, p. 7).

There were no significant results when testing the time between rice brand packing and purchase and the level of OTA in rice. As a result, it is assumed that this is related to the fact that rice bags were bought from reputable supermarkets with acceptable storage methods; yet, these bags were not vacuumed in general. Lane & Woloshuk (2017) showed that hermetic (airtight) storage bags effectively prevent the spread of fungi to non-infected kernels and block the impacts of external humidity changes. Furthermore, Tubbs et al. (2017) found that during the first few weeks of using hermetic bags, the oxygen concentration dropped to near zero at grain moistures of 18% and 20% moisture content.

Our results showed a significant difference ($p<0.001$) between the brands of both collections and OTA levels. This variability is related to discrepancies in production methods at processing and packing facilities, making rice more susceptible to OTA contamination. Inadequate temperature and humidity storage conditions and missing to discard rice grains that show signs of fungal infection can increase contamination levels (Ferre, 2016). Tang et al. (2019) found that rice samples with inadequate storage had a high probability of OTA accumulation. Furthermore, when fungi such as *Aspergillus* spp. and *Penicillium* spp. are suspected of being present, little can be done to prevent further accumulation of fungi, and that they can thrive over a temperature range of 10–40°C with the optimum temperature at a range of 25–35°C in the storage phase, and thus grains must be discarded (Mahuku et al., 2019).

For OTA, the tolerable daily intake is not considered a safety factor because the intake of those toxins must be kept as low as feasible. Accordingly, the Provisional Tolerable Daily Intake (PTDI) established by the Scientific Committee of Food (SCF) of the European Union was 5ng/kg body weight/ day (Raad et al., 2014). Based on the average OTA levels in our sample (0.42 μg/kg), the projected daily OTA exposure was 0.41 ng/kg body weight/day, which is lower than PTDI in EU. To our knowledge, no study has evaluated the Lebanese population's exposure to OTA from rice consumption. Raad et al. (2014) calculated an average dietary exposure to OTA of 4.28 ng/kg body weight/day in an adult urban population. Moreover, dietary OTA exposure from the rice in our sample was higher than that studied by González et al. (2006) (0.17 ng/kg b.w./day). In addition, after receiving a request from the European Commission, the European Food Safety Authority's (EFSA) Panel on Contaminants in the Food Chain (CONTAM Panel) assessed the risks to human health associated with the presence of ochratoxin A (OTA) in food. EFSA recently recommended calculating the margins of exposure by using the benchmark dose level (BMDL) (EFSA, 2020).

In contrast, the level of OTA exposure assessed in our study was lower than that reported in other countries. In Pakistan and Iran, for example, exposure to OTA from rice consumption was 4.2 and 0.62 ng/kg body weight/day, respectively (Iqbal et al., 2016; Rahimi, 2014).Although the results of different studies are valuable references, the comparisons should be carefully evaluated because the studies may differ in terms of methodology and model used to assess dietary exposure, types of rice and rice products, and the consumption patterns that might change between different regions and over time. As a result, even if rice is a significant source of OTA in one country, this may not be the case in other countries. Regarding Lebanon, Nasreddine et al. (2019) found that consumption of cereals, including refined grains like white rice, increased from 1997 to 2008/-2009. Even though the determined average daily exposure to OTA in our study is not exceptionally high, it is critical to keep the levels as low as possible to avoid the OTA harmful effects associated with increased rice consumption.

Our study's strengths include the fact that it was the first of its kind in Lebanon to assess the safety of packaged rice sold in the country in terms of OTA content, to determine OTA exposure levels from rice consumption, and to highlight the importance of including OTA MRLs in LIBNOR. In addition, we analyzed packed rice, as the majority of Lebanese people

purchase packed rice. Most of our samples were Lebanese brands; due to the Lebanese pound exchange rate issue, imported food products have become unaffordable for most Lebanese consumers, forcing a shift to purchase local and more cheap brands.

It is necessary to consider the study's limitations. First, the results contained some outliers. This could be attributed to a significant chance of false positive or negative results due to insufficient blockage of the surface of the microtiter plate immobilized with antigen, and future experiments should be done in triplicates to solve this. Due to financial constraints, we had to conduct the analysis twice. Furthermore, approximately half of the samples had no FSMS information. We were unable to contact all of the food companies and obtain clear responses because the majority of them were unwilling to cooperate, which is why we grouped them in the "absence/no information available" category. Last but not least, we were not able to measure the water activity due to technical issue with the water activity meter.

Additionally, around 20% of our samples lacked information on the production date. As a result, we were unable to examine the packing season effect of those brands on the degree of OTA, which may have masked the true association. Nonetheless, due to movement constraints imposed by the COVID-19 pandemic lockdown and road closures, we could not conduct our study on unpacked rice samples.

Furthermore, we could not detect an association between the time between packing and purchasing and the amount of OTA since the analyzed brands were purchased from highly reputable food stores with good storage conditions rather than diversifying them to include smaller food stores. Although ELISA is a reliable approach, HPLC is the gold standard for assessing OTA. Future studies must repeat the research using HPLC to validate the ELISA for identifying OTA in rice.

CHAPTER 6

CONCLUSION

In conclusion, rice is one of the world's most widely consumed foods. Rice contamination with OTA is common all over the world. Our research found that the rice samples we analyzed were within the EU limits. Parboiled rice had higher OTA levels than white and brown rice (not significant), and there was a significant difference across the two collections for the same brands. According to our findings, OTA contamination of rice sold in Lebanon is not currently a significant public health risk. Surveillance strategies and monitoring procedures, on the other hand, must be carried out regularly to ensure that rice is free of OTA contamination. It is recommended to purchase packaged rice brands with food safety management system certification from recognized food stores and appropriately store the rice in households to reduce the risk of OTA contamination.

Future research should analyze the level of OTA in unpacked rice sold in various areas and food markets around Lebanon to obtain a general understanding of the quality of rice purchased and consumed in Lebanon. Regular monitoring is required to account for smuggled and new brands accessing the Lebanese market. In addition to the moisture content, future research should also look at the *aw* of the grains, which is an essential indicator. Finally, ELISA results should be validated against the standard gold method of HPLC.

CHAPTER 7

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