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Ochratoxin A in Rice Marketed in United Arab Emirates: Occurrence and Exposure Level

By

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Dedication Page

To my beloved parents

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ABSTRACT

Rice is one of the most consumed staple foods around the world. *A. Circumdati*, *A. Nigri*, *P. verrucosum*, and *P. nordicum* can contaminate rice in subtropical and tropical hot and humid climates, which leads to mycotoxins secretion, like the carcinogenic ochratoxin A (OTA). Our study aims to investigate the amounts of OTA in packaged rice sold in United Arab Emirates (UAE) and the exposure to this toxin from rice consumption. A total of 127 packed white, parboiled, and brown rice bags were collected in two rounds, where 89 brands were identified. Out of 89 which, 38 were collected twice, while the remaining 51 brands were found in the market at either the first or second collection, but not both. OTA was measured using Enzyme-linked Immunosorbent Assay (ELISA) technique. OTA was detected in 127 out of 127 (100%) of the rice samples. The average concentration ± standard deviation of OTA was 0.29±0.08 μg/kg. Contamination ranged between 0.02 and 9.58 μg/kg. Moisture content in all rice samples was below the limit (14%). Only 2 samples (1.6%) had an OTA level above the EU limit (5 μg/kg). OTA in brown rice was higher than in white and parboiled rice, yet the difference was not significant. Packing season, packing country, and country of origin, did not have any significant effect. The presence of a food safety management certification resulted in a significant lower OTA level ($p=0.04$), while a significant difference was found between both collections for the same brands ($p=0.001$). Exposure to OTA from rice consumption was calculated as 0.2 ng/kg body weight/day

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

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List of Abbreviations

OTA: Ochratoxin A

FAO: Food and Agriculture Organization

FDA: US Food and Drug Administration

EFSA: The European Food Safety Authority

WHO: World Health Organization

EU: European Union

A.: *Aspergillus*

P.*: Penicillium*

a_w**:** Water activity

GAP: good agriculture practices

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

LC-FD: Liquid Chromatography with Fluorescence Detection

IAC: Immunoaffinity Chromatography

DLLME: Dispersive Liquid-Liquid Microextraction

FSMS: Food Safety Management System

Chapter One

Literature review

1.1 Mycotoxins:

1.1.1 Definition:

Filamentous fungi have a shown capability to form secondary metabolites. The accurate number of fungal metabolites is unknown, but it is estimated to have around 170,000 known natural products from fungal sources. Fungal metabolites can be classified into two categories, the first is pharmaceuticals such as penicillin or statins and the second is poisons such as aflatoxins or trichothecenes. Also, there is a category in between which is metabolites that are both toxic and pharmaceutically useful, such as the ergot alkaloids (Gruber-Dorninger, Novak, Nagl & Berthiller, 2016). The term "Mycotoxin" is used to refer to the compounds secreted as secondary metabolites by some filamentous fungi genera as Fusarium, Penicillium, Aspergillus and Alternaria. Moreover, mycotoxins are heat stable and naturally toxic compounds having a high bioaccumulation capacity (Khaneghaha, Fakhrib, Gahruiec, Niakousaric & Sant'Anaa, 2019).

In addition to producing toxicity in animals and humans, mycotoxins can exert phytotoxic or antimicrobial effects. The most important classes of mycotoxins include the highly carcinogenic aflatoxins (e.g., aflatoxin B1, AFB1), trichothecenes (e.g., deoxynivalenol, DON), fumonisins (e.g., fumonsin B1, FB1), ochratoxin A (OTA), and zearalenone (ZEN). These toxins, in addition to others, are controlled in many countries after comprehensive risk assessment, taking into consideration toxicity, occurrence, and consumption data as well as economic and political considerations (Gruber-Dorninger et al., 2016).

Mycotoxins create a threat for human and animal health, hinder global trading, waste foods and feeds, and redirect resources in the direction of research to regulate and lessen mycotoxin problems. Unfortunately, about 25% of the world's harvested crops are contaminated by mycotoxins annually, which leads to large agricultural and industrial losses that is estimated by billions of dollars (Alshannaq & Yu, 2017).

1.1.2 Factors leading to mycotoxin production:

Cereal products, fruits and vegetables can be contaminated by mycotoxins as a result of infection of the grain by fungi, or contamination taking place in the field either after harvest or during storage. Environmental Factors such as humidity, temperature, insect damage and drought play a major role in the variety and level of mycotoxin contamination. In addition to that, physical and chemical features of food such as pH, composition, and water activity, as well as the production management including storage, harvesting, processing conditions and tillage have an important impact on mycotoxin production (Khaneghaha et al., 2019). Water activity between 0.80 and 0.99 and temperatures between 25 and 30°C endorse fungal growth and, thus, increase the chances of mycotoxins presence (Ortiz et al., 2013). Also, animal products including meat, dairy and eggs can be contaminated with mycotoxins if the animal was supplied with contaminated animal feed (Shi et al., 2018).

However, good agriculture practices (GAP) have a significant influence on the reduction of mycotoxins contamination. As result of food processing, the final product could have different levels of mycotoxins, which in some instances can help decrease the contamination level (Khaneghaha et al., 2019). Inappropriate agricultural and harvesting practices and improper drying, handling, packing, storage, and transport procedures promote the growth of fungi, increasing the likelihood of mycotoxins formation (Marin et al., 2013; Marroquín-Cardona et al., 2014)

Mycotoxin contamination can happen pre-harvest when the crops are growing or post-harvest through processing, packaging, distribution, and storage of food products. It is known that all crops and cereals that are not stored well in high temperature and prompting humidity for a long period, will have mold growth and thus mycotoxin contamination (Alshannaq & Yu, 2017).

Several national and international public health and governmental establishments, such as the US Food and Drug Administration (FDA), World Health Organization (WHO), Food Agriculture Organization (FAO), and the European Food Safety Authority (EFSA), are giving more attention to mycotoxin contamination in food and feed. They are tackling this global problem by adopting strict regulatory

guidelines for major mycotoxin classes in food and feed. This resulted in creating limits on the occurrence of major mycotoxins in food and feed, in around 100 countries (Alshannaq & Yu, 2017).

1.2 Ochratoxins:

1.2.1 History of ochratoxin:

OTA was first discovered when it was isolated and chemically characterized in 1965. It was originally found in South Africa as a toxic metabolite of *Aspergillus ochraceus* in a corn meal that was purposely injected with this micro fungus. Thus, *Aspergillus ochraceus* was the first producer of OTA to be identified. OTA got its name from this fungus (ochraceus). Since its discovery in 1965, many studies have been done, which resulted in the formation of various mechanisms for OTA nephrotoxicity and carcinogenicity. This led the International Agency for Research on Cancer to classify OTA as a possible human carcinogen (group 2B) in 1993 based on several research and evidence on its possible carcinogenetic effect (Malir, Ostry , PfohlLeszkowicz, Malir & Toman, 2016).

1.2.2 Definition:

Ochratoxins are a group of secondary metabolites produced by fungi, such as *Aspergillus niger*, *Aspergillus ochraceus* or *Aspergillus carbonarius*, and by some *Penicillium* species (Zapasnik et al., 2021). OTA is a white, odorless, thermally stable, clear solid agent with low aqueous solubility, and it has the highest toxicity level among ochratoxin group. Furthermore, OTA is found in animal feed and in human food. Its presence is due to the weather conditions, and/or inappropriate storage of food. It is one of the most widespread contaminants in foods as cereals, coffee, wine, dried fruits and nuts, meat products, herbal medicines, food coloring agents and even in bottled water. Therefore, the elimination of OTA from the food chain is very hard due to its extensive presence as well as its heat stability (Melting point 168–173 °C). In general, the average concentration of OTA in products of plant origin is stated to range from 0.1 to 100 ng per gram. But those produced from wheat, oats, barley,

rye, maize, rice, millet, sorghum, soybeans, horse beans, peas, beans, broad beans, alfalfa, sunflower or pumpkin seeds, coconut, peanut cake and hay/silage have from 1 to 100 ng/g of OTA (Tao et al., 2018). Consumed OTA may seriously impair human health because of its nephrotoxic properties, carcinogenicity, teratogenicity, immunotoxicity, mutagenicity, and hepatotoxicity (Zapasnik et al., 2021).

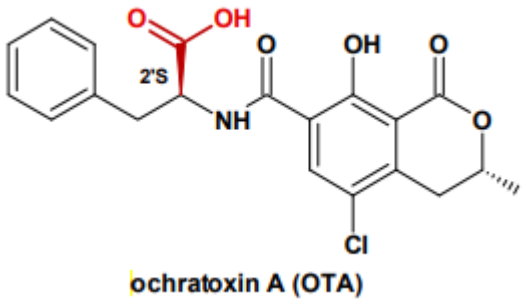

Figure 1: Chemical Structure of Ochratoxin A (Zapasnik et al., 2021).

1.2.3 Ochratoxin Metabolism:

OTA are absorbed from the stomach and the jejunum, but the specific mechanisms for these actions are not fully understood yet. Then, albumin binds OTA with a high affinity where 99.8% of OTA are albumin-bound in the human circulatory system. On the other hand, Erythrocytes contain only traces of OTA (Koszegi and Poór, 2016).

The tissue distribution of OTA is affected by several factors including the amount of toxin, the way of ingestion, the structure of the diet, and the overall health status of the individual. The major targets are the kidneys and the liver. Also, Skeletal muscles, fat tissues, and the brain contain OTA, but in lesser amounts (Kőszegi and Poór, 2016).

Some evidence suggests the susceptibility of the kidneys and liver might be because of their special transport mechanisms. In the kidneys, organic anion transporters (OATs) and in the liver, organic anion-transporting polypeptides (OATPs) are the major molecular structures responsible for OTA active cellular uptake (Kőszegi and Poór, 2016).

The major metabolic pathway of OTA involves hydrolysis, hydroxylation, lactone opening and conjugation (Tao et al., 2018). Previous studies have shown that most of OTA stays unchanged, and the liver is not the only organ to metabolize OTA. In the gut, Ochratoxin α (OTα) is the product of OTA hydrolysis where it is formed via the cleavage of the peptide bond of OTA by the action of proteolytic enzymes and by enzymes produced by the bacterial microflora. Another metabolic pathway of OTA is opening the lactone ring under alkaline conditions which results in the formation of extremely toxic compound called lactone-opened OTA (OP-OA) (Kőszegi and Poór, 2016; Tao et al., 2018). Also, human liver microsomes metabolize OTA into two epimers of 4-hydroxyochratoxin A (4-OH-OTA) that are 4(R)-4-hydroxyochratoxin A (4(R)-OH-OTA) and 4(S)-4-hydroxyochratoxin A (4(S)-OH-OTA) (Tao et al., 2018).

Finally, OTA metabolites are excreted through renal excretion in urine, fecal excretion in feces or through breast milk (Kőszegi and Poór, 2016).

The mechanism of action of OTA is very complex, but still unclear. One suggested mechanism is that OTA can induce cell apoptosis and inhibits protein and RNA synthesis. Many studies have found that free radicals are accumulated because of OTA exposure (Tao et al., 2018). OTA activates the caspase signaling pathway and cell apoptosis by generating an increase in NADPH and the P450 enzyme. Also, oxidative stress in the mitochondria and endoplasmic reticulum happens because of increasing reactive oxygen species (ROS). Oxidative stress hinders 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:

OTA can cause toxicity in the immune system, kidneys, and embryo in many species including human. kidneys have been shown to be the key target organ of OTA toxicity in most of the mammalian species (Erceg et al., 2019). It was shown in rodents that OTA intoxication damages various proximal tubule functions, including secretion of *para*-amino hippuric acid possibly via

disturbing the renal organic anion transporters. Yet, the effect of OTA on the activity of specific organic anion transporters in mammalian kidneys has not been studied. This renal transporter facilitates the transport of OTA and play an important role in OTA-induced nephrotoxicity development (Anzai et al., 2010). In addition to that, it has been shown that OTA is carcinogenic in variety of organs as kidney, liver, testes, urinary tract, bladder, skin and breast (Tao et al., 2018). Thus, it is classified by the IARC as a group 2B possible human carcinogen because of its hepatotoxic, teratogenic, immunosuppressive, nephrotoxic, and nephron-carcinogenic effect (Huang et al.,2020)

OTA was shown to induce the formation of reactive oxygen species (ROS), thus causing oxidative stress and ROS-mediated apoptosis, DNA adducts and DNA single-strand breaks. It also decreases glutathione (GSH) concentrations, which is a major antioxidant (Janik et al., 2020).

Effects on newborns are important to take into consideration. A study in Poland showed that OTA crosses the human placenta to fetal blood and that it is excreted into breast milk (Postupolski *et al*., 2006). OTA in offspring causes problems in brain development, including the cerebral cortex and hippocampus, neural tube defects, immune system problems and eye malformations. Not only in newborns, but adults as well can have neurological problems due to OTA exposure. It affects the macrophages, neutrophils, monocytes, T-cells, lymphocytes, and Natural Killer Cell activity. Thus, this will lead to chronic inflammation, with elevated IL-1b and IL-8. Moreover, variety of evidence have emphasized that OTA can be a possible cause for Balkan endemic nephropathy, a widespread, severe, progressive, and fatal kidney disease found in the Balkan countries (Erceg et al., 2019).

OTA is absorbed in the small intestine. Then, it enters the circulation to bind to the serum albumin in plasma. Almost all of the OTA (99.8%), in the human circulatory system, are found in the albumin-bond form. Red blood cells contain insignificant amounts of OTA. Post-absorption OTA is delivered to kidneys, liver, muscle, brain, and fat. Since kidneys are the major target of OTA, it was shown using transmission electron microscopy that OTA affects focal tubular

cell proliferation, multiple adenoma-like structures in layers of the renal papilla and in convoluted tubules. Moreover, OTA was present in bone marrow, skin, adrenal medulla, and cortex and myocardium (Janik et al., 2020).

Limits to OTA content have been set in different types of food. The European Union has set a maximum limit of ochratoxin A at 3 μg/kg for cereal products, 5 μg/kg for unprocessed cereals, 10 μg/kg for dried fruits, and 15 μg/kg for spices, 5 ng/kg in coffee beans, 10 ng/kg in instant coffee, 0.5 μg/kg in cereal-based food for infants and children, and 2 μg/kg in wines. Additionally, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) has stated that the maximum tolerable consumption of OTA is 0.1 μg/kg of body weight per week (Janik et al., 2020).

1.3 Rice:

1.3.1 Rice varieties:

Rice, wheat, and maize are the most common food crop worldwide. They provide more than 42% of all calories consumed by the entire human population. Rice is the most important food crop for people in low- and lower-middle-income countries. It is the staple food of more than 50% of the population globally where around 3.5 billion people depend on rice in more than 20% of their daily caloric intake. (Global Rice Science Partnership, 2013)

Rice provides more than 20% of calories consumed worldwide, especially in East and South Asia, Middle East, West Indies and Latin America. It is grown in more than100 countries with 90% of the total global production from Asia. Although that after post-harvest processing, there is a huge variety of rice that can reach more than 110,000 cultivated varieties that differ in quality and nutritional content, rice can be classified as either white or brown (Fukagawa and Ziska, 2019).

The scientific name for rice is *Oryza*. *Oryza sativa* is the most common species and is categorized into the long-grain *indica*, and short-grain *japonica*. (Harvard T.H. Chan School of Public Health, 2021). Rice varies in grain size, color,

stickiness, aroma, thickness and growing conditions/production practices that impact quality and nutritional profiles. The global market of rice of different varieties is influenced by regional and cultural preferences (Fukagawa and Ziska, 2019).

1.3.1.1 Shape of the grain*:*

The shape of the rice grain may be long, short, or medium. This refers to the length and width of the rice grain post cooking:

- *Long grains* have a slender kernel over four times as long as they are wide. Upon cooked, long grain rice remains differentiated, separated and fluffy. Jasmine and Basmati rice are examples of long grain rice (Harvard T.H. Chan School of Public Health, 2021)
- *Medium grains* have a shorter and wider kernel than long grain. This yields a soft and semi-sticky consistency when cooked. Arborio rice is an example of medium grain (Harvard T.H. Chan School of Public Health, 2021)
- *Short grains* have a kernel only twice as long as they are wide, and their texture when cooked is the stickiest. This rice is used in sushi for example (Harvard T.H. Chan School of Public Health, 2021).

1.3.1.2 Processing and nutritional value of the grains:

Many studies on nutrient content of rice have showed that the nutritional value of the rice is related to the number of factors like strain of rice, such as white, brown, red, and black, quality of the soil and its nutrients where the rice is planted, processing methods and preparation methods prior to consumption (Kalati, Gohain & Hazarika, 2021).

The rice grain consists of 3 parts: the hull, the outer protective covering and the rice caryopsis or fruit. After de-hulling the rice contains outermost pericarp followed by seed coat, nucellus and aleurone layer while the embryo and the endosperm are lying inside (Kalati, Gohain & Hazarika, 2021).

Each layer has its own components, for example endosperm contains carbohydrates, protein and some B vitamins; embryo and aleurone cells contain higher amount of protein and lipid while pigment for the color is only found in the pericarp (Kalati, Gohain & Hazarika, 2021).

These layers are either lost or retained depending on the processing methods used. Therefore, different processing methods yields rice that differs in its nutrient content. For instance, the brown rice has all its part retained (the bran, germ, and the endosperm) where only the outer hull is taken off. The kernel of the brown rice is rich in proteins, vitamins, minerals, and fiber; thus, it is considered to have a low "glycemic index". The brown rice is changed into white rice by 100% milling and this process destroys vitamin B3 by 67%, vitamin B1 by 80%, vitamin B6 by 90%, minerals such as manganese, phosphorus, iron and all of the dietary fiber and essential fatty acids (Kalati, gohain & Hazarika, 2021).

White rice is consumed more than the brown one. This is because of various reasons, including cooking easiness, palatability and taste, and shelf life. Also, white rice has a higher glycemic load and may affect glucose homeostasis; thus, it can be served with other foods so it can be considered part of a “healthy” plate (Fukagawa and Ziska, 2019). Regarding the calorie content of rice, it varies depending on each type. For example, 1 cup of cooked medium-or short-grain white has 241.8 kcals, while 1 cup medium-grain brown rice has 218.4 kcals. Also, 1 cup long-grain brown rice contains 216.5 kcals, and regular long-grain white rice contains 205.4 kcals (Ricepedia, 2018).

As the global population is increasing, rice will still be a staple food. However, it will be essential to encourage on consuming brown rice and to find ways to retain the phytonutrients that are lost during refining (Fukagawa and Ziska, 2019).

1.3.2 Sources:

Rice is grown in 113 countries, for that it is the staple food for over half the world's population. The cultivation of rice is the major source of income for about 100 million households in Asia and Africa. Almost four-fifths of the world's rice is produced by small-scale farmers and is consumed locally (FAO, 2004).

The cultivation of rice occurs in subtropical and tropical warm and humid areas, mainly in Asia. It has aquatic characteristic; thus, it is harvested at elevated moisture levels (35-50%). Then, rice is dried and stored. Since rice is grown in flooded irrigation conditions and high levels of moisture, it is prone to get contaminated by mold and mycotoxin infection. Moreover, improper storage and climatic conditions, such as floods and heavy rainfall at harvest time, exacerbate the situation. Most farmers sun-dry the rice, but this way is ineffective to decrease moisture to desirable levels; therefore, rice will be more susceptible for fungal growth and contamination (Majeed et al., 2018). Drying of the rice grain to less than 14% of moisture content limits fungal growth and colonization during storage (Mutiga et al., 2021).

Despite the health benefits of rice bran, it is often prone to mycotoxin contamination due to the growth of toxin-producing fungi at the aleurone layer. These fungi can be *Aspergillus flavus*, *Aspergillus, Fusarium,* and *Penicillium*. The presence of mycotoxins in food, including rice bran, is a major health problem, and actions should be taken to control and minimize exposure. Thus, it is essential to have a fast, sensitive, strong, and cost-effective technique to detect the presence of mycotoxins in rice bran (Salim, Sukor, Ismail & Selamat, 2021). 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). Annually, 15% of produced rice is wasted because of parasite contamination and other potentially harmful species that commonly occur due to poor storage conditions (Ruadrew et al., 2013).

Rice is contaminated in OTA in many developed and developing countries as Canada, Ireland, Greece, United Kingdom, Portugal, and Pakistan (Iqbal, Asi, Hanif, Zuber, & Jinap, 2016).

1.3.3 Production and consumption pattern:

From 1960 to 1999, the worldwide consumption of rice increased 2.5 times. Around 89% of the global rice production is in eastern Asia, particularly in China and India (FAO, 2004). Statistics predicted that global rice consumption will probably rise from 439 million tons (milled rice) in 2010 to 555 million tons in 2035 (Global Rice Science Partnership, 2013). The major increase will be in Asia, which will account for 67% of the total rise, rising from 388 million t in 2010 to 465 million t in 2035. Also, 30 million tons of rice will be needed by Africa, which is a rise of 130% compared to rice consumption in 2010. In the Americas, total rice consumption is estimated to increase by 33% by 2035 (Global Rice Science Partnership, 2013). According to the USDA, worldwide consumption of rice was 501,969 thousand metric tons in January 2021 (USDA, 2021).

China and India account for more than 50% of the world's rice consumption. Bangladesh, the Lao People's Democratic Republic, Cambodia, Vietnam, Myanmar, Thailand, Indonesia, and the Philippines reported intakes are >110 kg per capita yearly. Also, high consumption of rice has been reported in Latin America and Caribbean countries, as Guyana, Suriname, Cuba, Panama, Costa Rica, Peru, Ecuador, and Nicaragua. In South America, typical consumption of rice is 45 kg per capita yearly, whereas it has increased to 70 kg per capita yearly in the Caribbean. Rice consumption is on the rise as well in the Pacific Island countries of the Solomon Islands, Vanuatu, and Fiji (Muthayya, Sugimoto, Montgomery, &. Maberly, 2014).

As seen before the rice consumption is not uniform in all countries. As urbanization increases, rice consumption decreases. For instance, Japan consumes 65 kg per capita whereas Bangladesh consumes 258 kg which is

around four times higher (Ali, 2019). The top ten countries in terms of rice production and consumption are mainly located in Asia and are shown in the figure 2 below.

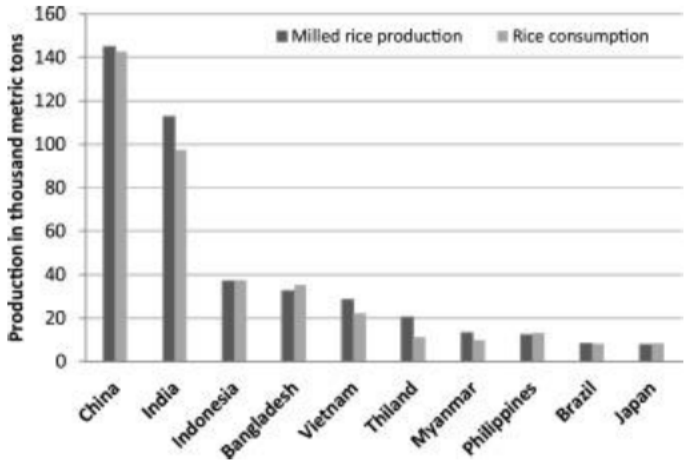

Figure 2: Rice production and consumption in top ten countries in the world in 2017/2018 (Shahbandeh, 2021).

Moving to the Middle East and North Africa regions, main rice producers are Egypt, Turkey and Iran. Unfortunately, similar to other products, production falls behind the consumption in the region. From 2011 to 2013, the MENA region consumed 13 million tons of rice annually where more than 50% were imported. Figure 3 below elucidates the rice consumption in MENA region. (Nigatu & Motamed, 2015).

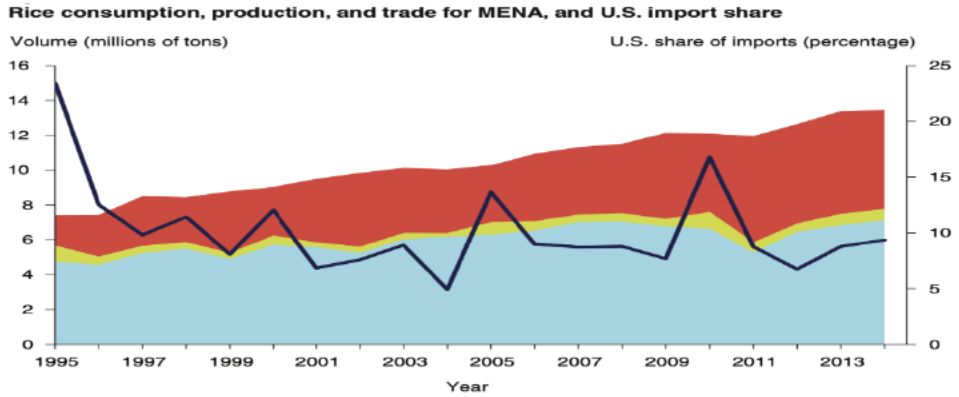

Figure 3: Rice consumption, production, and trade for MENA, and U.S. import share (Nigatu & Motamed, 2015).

1.4 Ochratoxin A (OTA) in rice:

1.4.1 Analytical methods:

It is essential to have a rapid, sensitive, robust, and cost-effective technique to detect the presence of OTA in rice (Salim et al., 2021). Analytical methods to determine OTA include instrument-based methods like high-performance liquid chromatography (HPLC) connected to a fluorescence detector (HPLC/FLD), gas chromatography-mass spectrometry (GC-MS), and liquid chromatography/mass spectrometry (LC/MS) (Salim et al., 2021; Huang et al., 2020).

HPLC have a high sensitivity, specificity, accuracy, easy usage, and reproducibility of the obtained results, but it is costly and needs trained staff (Iqbal, Asghar, Ahmed, Khan, & Jamil, 2014). Additionally, LC-MS/MS it is a combination of HPLC techniques and mass spectrometry method. This improves the ability to detect OTA, providing selectivity and sensitivity. Same as HPLC, this method is expensive and needs highly trained experts. (Iqbal et al., 2014). Also, it has been widely used in the determination of mycotoxins in various complex matrices due to its high sensitivity and ability to detect multiple mycotoxins in a single analysis. LC-MS/MS is the most favorable instrumental method for multi-mycotoxin detection. It is highly selective and can perform screening, confirmation, and quantitation of several analytes simultaneously. The pre-treatment procedure of the sample is essential to improve the sensitivity and selectivity of the analytical method (Salim et al., 2021).

Although these methods offer precision and reliability, but they have some limitations as mentioned above which include high cost, requiring a certain level of expertise to use them, and not being appropriate for on-site use (Huang et al., 2020).

Another method is Thin-layer chromatography (TLC), where this technique differs from the prior ones by being cheap in cost, but the previous ones (HPLC,

LC–MS/MS) are more advanced. Unfortunately, TLC provides inadequate sample clean-up, which causes an incomplete division, reduced sensitivity and may lead to false results (Iqbal et al., 2014).

Newer techniques, like immunoassays, have emerged to overcome the above limitations. They work as a substitute for instrument-based methods. Immunoassay-based techniques are based on the binding of antigen to antibody. In addition to that, they are cheap, simple, and sensitive. One example of immunoassays is enzyme-linked immunosorbent assay (ELISA). This technique is used easily and have high sensitivity, adaptability, high-throughput minimal sample extraction and sample volume need. Also, the quantitative analysis is performed by an intermediate, which is a simple and rapid kit that that shows similar results to TLC and HPLC. However, it needs delicate supervision for each test to obtain accurate results (Iqbal et al., 2014; Pereira, Fernandes, & Cunha, 2014).

Another technique is fluorescence polarization immunoassay (FPIA) method that has a better sensitivity. In FPIA, apart from the purification procedure, the whole analytical process needs 10 minutes to be completed showing its practical applicability as a rapid screening method (Salim et al., 2021; Huang et al., 2020).

In recent years, dispersive liquid-liquid microextraction (DLLME) has emerged as a contemporary technique in sample preparation. It is simple, effective, and considered a green microextraction technique. This is because it employs a minimal volume of solvents and a mixture of organic solvents that act as extraction and dispersive solvents (Salim et al., 2021).

1.4.2 Reported OTA levels in rice worldwide:

Table 1 summarizes the findings of studies on OTA worldwide:

Table 1: OTA levels in rice worldwide

Country	Year of publication	Sample size (rice samples)	Types of rice	Analytical method	Reported OTA level as µg/kg (mean± SD as µg/kg)	Exposure level as ppb or ppm or µg or ng /kg body weight/day	Reference
Kenya	2021	200	White rice	LC–MS/MS ELISA	0-111	-	Mutiga et a
Iran	2017	220	Rice	ELISA	3.51± 2.43	8.36	Habib et al
Iraq	2020	21	Rice	ELISA	5.1-12.7	-	Alhendi et
Belgium	2020	14	Rice	LC-MS/MS	0.09±0.30	0.3	Meerpoel e
Korea	2005	60	Polished rice	IAC HPLC-FLD	1	-	Park et al.
Malaysia	2013	50	Red rice	IAC; ELISA	0.90±0.57	-	Samsuddin al.
Iran	2016	308	Rice	ELISA	3.60±2.66	0.62	Rahimi
Canada	2011	100	white, brown, red, black, basmati, jasmine and wild	LC-MS/MS	0.49(0.12)	-	Bansala et
Brazil	2012	230	rice with the processing fractions (bran, rice husk and broken)	IAC, HPLC-FD	0.20-0.24	-	Almeida et

Pakistan	2016	208	White, brown rice and rice products	HPLC-FD	White rice: 8.50 ± 0.60 Brown rice: 7.84 ± 0.90 Rice flour: 4.91 ± 1.53 Sweet puffed rice balls: 3.87 ± 0.75 Rice cookies: 3.18 ± 0.60 Rice sweets: 5.10	24.2-24.7	Iqbal, Asi, Hanif, Zub & Jinap
Tunisia	2008	209	Rice	ELISA	3.5 ± 5.3	-	Ghali, Hmaissia-khlifa, Ghorbel, Maaroufi, & Hedili,
Vietnam	2007	100	Rice	HPLC-FD	2.78 (0.75)	23	Nguyen, Tozlovanu, Tran, & Pfo Leszkowicz

HPLC: High-Performance Liquid Chromatography; HPLC-FD: High-Performance Liquid Chromatography with Fluorescence Detection; LC-MS/MS: Liquid Chromatography Tandem Mass Spectrometry; ELISA: Enzyme-Linked Immunosorbent Assay; IAC: Immunoaffinity Chromatography

Chapter Two

Aims and Hypotheses

2.1 Gaps in Literature:

Until now, no study was performed in United Arab Emirates (UAE) to assess the safety of rice marketed in the country, in terms of OTA content, and to determine the exposure levels of UAE inhabitants to OTA from the rice consumption.

2.2 Research objective and significance:

Rice is an essential ingredient in the Mediterranean cuisine and on the Arabic table. Rice is imported to UAE as pre-packed, or unpacked and then, packed inside the country or sold as unpacked. Also, UAE started to grow its own rice since May 2020, but still the majority of the consumption is from imported rice. This fact makes the rice supply in UAE more susceptible to contamination with OTA due to high humidity and temperature during transportation and storage. Therefore, the objective of our study is to assess the quality of rice marketed in UAE and determine the exposure of the population in terms of OTA from the consumption of rice. In addition to that, the effect of the weather and season, presence of packaging, type of rice, and existence of a food safety management system certification will also be assessed. Since no Emirati standard for OTA in rice has been implemented yet, our work will help UAE authorities by paving the way for the development of MRLs.

2.3 Hypotheses:

H1: Seasonal effect: Warm and humid packing seasons will enhance the production of OTA in rice compared to cold and dry seasons. According to the literature, the favorable production conditions for the ochratoxin A are the optimum temperature 25 ºC, a_w >0.95 and moisture content 18-22% (Atumo, 2020).

H2: Presence of certification: Rice brands with food safety management system certification (ISO22000, HACCP, FSSC22000 …) will tend to be less contaminated with OTA due to the conforming storage conditions and quality control practices.

H3: Type of rice: Brown rice will tend to be more contaminated with OTA than white rice, since upon processing brown rice to white rice, the hull, the germ, and the bran layer of the rice along with the fungi and OTA will be removed during this procedure.

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 temperature may increase, 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 to have higher levels of OTA compared to rice packed in developed countries because of the difference in 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 developed countries because of the absence of good agricultural practices (antifungal pesticides, post-harvest storage conditions) in the former.

*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 Three

Materials and methods

3.1 Sample collection:

Screening of the Emirati market in Abu Dhabi for white, parboiled and brown rice brands was done. Afterwards, rice was collected from retailers in different areas over two production dates and two seasons (March 2021 and June 2021). During first collection in March (winter), 62 packaged rice brands were collected from stores across Abu Dhabi. During second collection in June (summer), 65 packaged rice brands were collected from stores. In total, 89 brands were identified, and out of them, 38 were collected twice, while the remaining 51 brands were found in the market at either the first or second collection, but not both. Rice bags were stored at -18 degrees until analysis was done.

Screening of OTA was performed using the ELISA technique.
Therefore, the independent variables included:

- Seasonal effect since OTA production is influenced by temperature and humidity.
- Presence of a food safety management system certification (ISO22000, HACCP, FSSC22000 …), since this indicates the presence of better storage conditions and quality control practices.
- Type of rice (white vs. brown) since brown rice tends to have higher OTA levels compared to white rice. This is because the latter experienced removal of the exterior or bran and thus fungi and their OTA were eliminated in the process.
- Time (in weeks) between the rice production or packaging date and purchase date from retailers, which reflects the storage conditions of rice bags in the stores because storing in a dry environment decreases the risk of contamination.

- Country of packaging of rice represents the quality of rice storage and post-harvest practices.
- Country of origin of rice reflects 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 (long vs short/medium).

3.2 Sample preparation:

The rice samples were stored in a freezer (-18ºC) and protected against sunlight. Sample preparations took place at LAU Beirut's laboratories at room temperature (20 - 25 °C). First, a representative sample was ground and thoroughly mixed before moving to the extraction procedure. After grinding, 7g (6.95-7.04 g) were weighed from each sample. As per the ELISA kit manufacturer instructions (RIDASCREEN Ochratoxin A 30/15R1312, r-biopharm, Germany), 50 ml of ECO extractor were diluted in 500 ml of distilled water (1:10 dilution) for the extraction process. After that, the weighed 7g of ground rice 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 for 5 minutes at 3.500 g and at room temperature of 20 - 25 °C. Then, buffer salt sachet was dissolved in 1 L of distilled water and shaken well to dissolve. After that, 1 ml of wash buffer solution was added to new tubes with 1 ml of the supernatant using different pipette tips for each tube. Finally, 50 µl of the diluted supernatant solution was added to each well to perform the test.

3.3 Sample Analysis by ELISA:

This process was done quickly to avoid the dry up of the microwells. First, wells were added to the microwell holder for all standards and samples to be assessed. Locations of standards and samples were recorded. Next, 50 µl of the standard or prepared sample was pipetted into separate wells using a different pipette tip for each standard or sample. Then, 50 µl of enzyme conjugate was added to the bottom of each well, and the plate was gently shaken manually to mix the reagents and was then incubated for 30 min at room temperature (20 - 25 °C) in a

dark place. Furthermore, the liquid was poured out of the wells and the microwell holder was tapped upside down vigorously three repeated times 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 again to eliminate any residue of the liquid. This washing procedure was repeated two more times.

In addition, 100 µl of substrate/chromogen was added to each well. Then, plate holding the mixture was manually shaken gently and incubated for 15 min at room temperature (20 - 25 °C) in the dark. Finally, each well was filled with 100 µl of stop solution to stop any reactions. Lastly, the plate was gently shaken manually, and then absorbance was measured at 450 nm by a plate spectrophotometer within 15 minutes of adding the stop solution, and reading was done.

OTA concentration estimation relies on creating the standard curve, which is based on the absorbance of known concentration of 6 OTA standards (0, 0.03, 0.1, 0.3, 1, and 3 µg/L). Absorbance values of standards and samples were entered in a special software named the RIDASOFT® Win.net (Art. No. Z9996) that is available for evaluation of the RIDASCREEN® enzyme immunoassays, which will show the OTA concentrations [µg/kg].

3.4 Moisture content analysis:

3.4.1 Quality of rice and moisture content:

The quality of the rice, which includes its moisture content, is precisely related to fair trade since prices or acceptance criteria are defined based on it. Rice quality is perceived differently based on several aspects, among customers from diverse regions, countries, cities, and urbanization levels. For example, in South Asia, the quality is specified by the physical characteristics of the grains, satiety, and smell. On the other hand, in Southeast Asia, best quality is defined by nutritional value, softness, and aroma. One variable that affects the quality of the rice is the

moisture content, especially if it has been stored for a long period of time (Custodio et al., 2019)

Various sources have presented several feasible standardized air-oven methods to measure the moisture content of rice. It was based on drying the whole or ground grains in the oven for a certain period of time. One of the standard approaches is by the "Association of Official Analytical Chemists (AOAC), 1980." (Chen, 2003).

3.4.2 Moisture Content Analysis: Association of Official Analytical Chemists (AOAC):

The moisture content analysis of the first and second collection took place during November 2021. The first collection's 62 rice samples and the second collection's 65 rice samples were studied at the laboratory at LAU Byblos. All samples were previously ground at LAU Beirut's laboratory throughout the sample preparation phase for the ELISA analysis. Two grams of the well-mixed rice sample were weighed and added to cooled and weighed crucible. The weight of each crucible containing the sample was recorded. Then, the air oven was heated and retained at a temperature of 130±3°. After that, the crucibles holding the rice samples were put in the air oven for 1 hour for drying. After one hour, the drying was complete, and the crucibles were removed from the oven. The weighing of the crucibles again took place as soon as they cooled down and reached room temperature. Finally, 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 calculated on a wet basis (wb) after using the oven drying procedure (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 International Rice Research Institute, the moisture level of the rice grains should be less than 14% of the weight (IRRI, n.d.).

3.5 Determination of Exposure to OTA From Rice Consumption In UAE:

Exposure level to OTA from rice consumption can be calculated by multiplying the average OTA concentration detected in the samples tested by the average rice consumed in UAE, and then dividing by the average body weight of Emirati population, as follows:

Exposure (ng/kg body weight/day) =

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

The average consumption of rice in UAE is 56.1 kg per capita (FAO, 2020). Also, the average body weight in UAE is 76 kg (UAE Ministry of Health and Prevention, 2018)

Chapter Four

Statistical Analysis

OTA 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. Continuous variables are presented as means and standard deviations; whereas categorical variables are demonstrated by percentages. Bivariate analysis was conducted to determine associations between explanatory (independent) variables (effect of production date, effect of country of origin, effect of rice type, and effect of food safety system certificate), and the levels of OTA considered as dependent variable. Also, multivariate analysis (multiple linear regression) was applied to study further the associations between the independent and dependent variables, after adjusting for covariates. Mean and standard deviations 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 ANOVA F test for more than two groups. When the ANOVA F test showed statistical significance, post-hoc analysis was carried out using the Bonferroni correction for pair-wise comparisons, which corrects the family-wise type I error. All analyzes were carried out at the <0.05 significant level.

Chapter Five

Results

5.1 OTA content in rice samples:

OTA was detected in 127 out of 127 (100%) of the rice samples tested. Average concentration values of OTA for each brand from collection 1 and 2 are presented in **Tables 2 and 3**. Overall average (± standard deviation) of OTA in the 127 rice samples was 0.29±0.08 µg/kg.

The level of contamination ranged between 0.02 and 9.58 µg/kg. Only 2 samples out of the 127 brands (1.6%) had an average level above the European Union (EU) limit (5µg/kg).

Table 2: OTA (mean±SD) content in rice samples from collections 1 and 2

COLLECTION 1 (N= 62)		**COLLECTION 2 (N= 65)**	
Sample	**Average OTA ± SD (µg/kg)**	sample	**Average OTA ± SD (µg/kg)**
1a	0.07±0.02	5b	0.14±0.03
2a	0.53±0.37	6b	0.14±0.02
3a	0.09±0.02	7b	0.54±0.06
4a	0.06±0.02	8b	0.07±0.01
5a	0.06±0.01	9b	0.08±0.00
6a	0.41±0.28	10b	0.07±0.01
7a	1.03±0.08	12b	0.12±0.05
8a	0.10±0.08	13b	0.08±0.01
9a	0.15±0.01	14b	0.08±0.03
10a	0.13±0.10	15b	0.11±0.00
11a	0.08±0.01	16b	0.05±0.02
12a	0.14±0.06	17b	0.03±0.01
13a	0.20	19b	7.73±0.20
14a	0.26±0.18	22b	0.10±0.01

15a	0.07±0.03	23b	0.06±0.02
16a	0.06±0.06	25b	0.24±0.04
17a	0.08±0.02	26b	0.02±0.01
18a	0.39±0.36	27b	0.07±0.02
19a	0.35±0.07	28b	9.58±0.56
20a	0.10±0.05	29b	0.04±0.02
21a	0.08±0.02	30b	0.04±0.01
22a	0.29±0.28	31b	0.02±0.01
23a	0.04±0.00	33b	0.06±0.03
24a	0.02±0.01	34b	0.12±0.05
25a	0.04±0.01	36b	0.03±0.01
26a	0.10±0.10	37b	0.07±0.01
27a	0.99±0.00	39b	0.07±0.04
28a	1.31±0.38	40b	0.22±0.02
29a	0.12±0.01	41b	0.04±0.00
30a	0.43±0.31	43b	0.05±0.01
31a	0.02±0.01	45b	0.04±0.03
32a	0.04±0.01	46b	0.03±0.01
33a	0.08	49b	0.08±0.04
34a	0.88±0.74	53b	0.10±0.01
35a	0.07±0.05	54b	0.04±0.01
36a	0.04±0.02	55b	0.03±0.03
37a	0.08±0.08	57b	0.04±0.03
38a	1.24±0.81	58b	0.09±0.04
39a	0.07±0.05	64b	0.02±0.01
40a	0.17±0.06	65b	0.02±0.01
41a	0.03±0.02	66b	0.11±0.04
42a	1.02±0.96	67b	0.09±0.02
43a	0.04±0.04	68b	0.05±0.02
44a	0.03±0.00	69b	0.05±0.04

45a	1.05±1.40	70b	0.09±0.01
46a	0.05±0.04	71b	0.05±0.01
47a	0.15±0.04	72b	0.11±0.09
48a	0.03±0.00	74b W	0.05±0.02
49a	0.21±0.08	74b Br	0.04±0.00
50a	0.03±0.00	75b	0.04±0.01
51a	0.15±0.03	76b	0.10±0.04
52a	0.12±0.04	77b	0.07±0.01
53a	0.08±0.07	78b	0.07±0.04
54a	0.03±0.01	79b	0.12±0.04
55a	0.15±0.02	80b	0.10±0.03
56a	0.08±0.01	81b	0.05±0.00
57a	0.13±0.07	82b	0.03±0.01
58a	0.15±0.04	83b	0.11±0.01
59a	0.16±0.01	84b	0.11±0.05
60a	0.20±0.03	85b	0.09±0.00
61a	0.14±0.08	86b	0.28±0.02
62a	0.08±0.01	87b	0.07±0.02
		88b	0.10±0.05
		89b	0.11±0.01
		90b	0.03±0.02

Table 3: Minimum, Maximum, & average mean (µg/kg) of OTA in collection 1 (N=62), collection 2 (N=65) and all samples combined (N=127)

	Minimum (µg/kg)	**Maximum (µg/kg)**	**Average Mean (µg/kg)**
Collection 1 (N=62)	0.02	1.31	0.23
Collection 2 (N=65)	0.02	9.58	0.35

All samples combined (N=127)	0.02	9.58	0.29

5.2 Moisture analysis (%) in rice samples:

Table 4. 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 4: Moisture content (%) in rice samples from collection 1 and 2

COLLECTION 1 (N= 54)		**COLLECTION 2 (N= 51)**	
Code	**Moisture Content (%)**	**Code**	**Moisture Content (%)**
1a	12.50	5b	9.45
2a	12.50	6b	9.00
3a	11.00	7b	10.00
4a	11.00	8b	11.50
5a	10.00	9b	12.50
6a	10.00	10b	10.95
7a	10.00	12b	9.00
8a	12.50	13b	8.46
9a	11.00	14b	10.05
10a	11.56	15b	10.95
11a	10.00	16b	10.00
12a	11.44	17b	10.00
13a	11.50	19b	10.45
14a	10.00	22b	
15a	10.50	23b	10.05
16a	10.50	25b	9.50
17a	11.50	26b	11.44

18a	10.00	27b	7.46
19a	11.00	28b	9.45
20a	10.50	29b	9.55
21a	13.93	30b	8.54
22a	11.39	31b	10.00
23a	11.94	33b	9.00
24a	9.95	34b	10.00
25a	9.50	36b	9.50
26a	11.06	37b	9.50
27a	10.50	39b	10.50
28a	11.00	40b	10.00
29a	11.56	41b	9.45
30a	11.06	43b	10.50
31a	11.94	45b	8.04
32a	10.50	46b	7.54
33a	10.50	49b	7.46
34a	10.55	53b	8.96
35a	10.45	54b	11.44
36a	11.00	55b	12.94
37a	10.95	57b	7.96
38a	10.50	58b	11.94
39a	11.94	64b	10.00
40a	8.96	65b	9.50
41a	9.95	66b	8.46
42a	11.00	67b	10.00
43a	11.00	68b	10.00
44a	9.00	69b	8.00
45a	9.95	70b	10.95
46a	9.50	71b	10.00
47a	9.45	72b	9.00

48a	9.55	74b W	10.95
49a	11.00	74b Br	10.00
50a	12.00	75b	10.05
51a	10.00	76b	8.96
52a	10.55	77b	9.50
53a	10.00	78b	10.89
54a	10.50	79b	11.50
55a	10.95	80b	10.45
56a	10.00	81b	12.06
57a	7.04	82b	11.00
58a	12.00	83b	11.06
59a	10.00	84b	9.55
60a	11.44	85b	10.50
61a	11.06	86b	11.00
62a	10.45	87b	10.45
		88B	12.50
		89b	9.00
		90b	11.00

5.3 Effect of rice type on OTA levels in rice:

No significant difference was found between white /parboiled and brown rice (p=0.346) (**Table 5**).

Table 5: Effect of rice type on OTA

Rice Type [b]	N= 127	Mean	SD	p-value
White/ parboiled rice	97	0.15	0.24	
Brown rice	28	0.20	0.26	0.346

* All data are presented as N and mean (±SD). Difference in rice type between white/parboiled and brown was tested: [b] ANOVA F.

5.4 Seasonal effect of rice packing in UAE 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 UAE (p= 0.62) (**Table 6.**).

Table 6: Seasonal effect of rice packing in UAE on OTA

Packing season (UAE as country of packing)	N= 103	Mean	SD	p-value
Fall/Winter	74	0.15	0.25	0.62
Spring/Summer	51	0.17	0.24	

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

5.5 Effect of country of packing on OTA levels in rice:

No significant difference was found between rice brands packed in UAE compared to those packed in other countries (p= 0.973) (**Table 7.**).

Table 7: Effect of rice packing country on OTA

Country of packing [a]	N= 103	Mean	SD	p-value
UAE	39	0.16	0.20	
Other countries	77	0.16	0.26	0.973

* All data are presented as N and mean (±SD). Difference in country of packing between UAE and other countries was tested: [a] Independent t Test.

5.6 Effect of country of origin on OTA levels in rice:

No significant difference was found between rice from Developing countries (China, India, Pakistan, Thailand) and Developed countries (USA, Italy), in terms of country of origin (p= 0.41) (Table 8.).

Table 8: Effect of country of origin on OTA

Country of origin[b]	N= 103	Mean	SD	p-value
Developing (India, Pakistan, Thailand, China)	111	0.17	0.26	0.41
Developed (USA, Italy)	14	0.11	0.06	

* All data are presented as N and mean (±SD). Difference in country of origin between Developing countries (India, Pakistan, Thailand, and China) and Developed countries (USA, Italy) was tested: [b] ANOVA F

5.7 Effect of a food safety management system on OTA levels in rice:

There was a significant difference between rice brands packed in facilities that had a food safety management system and those that did not have one or did not have/present any related information. (p= 0.04) (**Table 9.**)

Table 9: Effect of a food safety management system on OTA

Food safety management system[a]	N= 103	Mean	SD	p-value
Presence	58	0.11	0.14	0.04
Absence/ Information not available	67	0.20	0.30	

* 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: [a]Independent t Test.

5.8 Effect of grain size on OTA levels in rice:

There was no significant difference between long rice grain and short/medium rice grain (p= 0.175). (**Table 10**).

Table 10: Effect of grain size on OTA

Grain size[a]	N= 103	Mean	SD	p-value
Long	73	0.18	0.28	
Short/ Medium	52	0.13	0.19	**0.175**

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

5.9 Effect of time between packing and purchasing on OTA levels in rice:

There was a significant difference found between packing and purchasing times of rice bags (p= 0.037) (Table11).

Table 11: Effect of time between packing and purchasing of rice bags on OTA

Time between packing and purchasing[b]	N	Mean	SD	p-value
1 to 9 weeks	9	0.03	0.11	
10 to 19 weeks	46	0.07	0.27	0.037
20 to 29 weeks	29	0.17	0.04	
30 weeks and above	41	0.21	0.30	

* All data are presented as N and mean (±SD). Difference intime between packing and purchasing between 1 to 9 weeks, 10 to 19 weeks, 20 to 29 weeks and 30 weeks and above was tested: [b] ANOVA F.

5.10 Effect of collection on OTA level levels in rice:

A significant difference was found between both collections of rice bags (p<.001) (**Table 12**).

Table 12: Effect of collection on OTA

Collection[b]	N	Mean	SD	p-value

First	62	0.24	0.32	
Second	63	0.09	0.08	0.001

* All data are presented as N and mean (±SD). Difference between first and second collections was tested: [b] ANOVA F.

5.11 Exposure to OTA From Rice Consumption In UAE:

The calculated daily exposure to OTA was 0.2 ng/kg body weight/day.

Chapter Six

Discussion

Until today, to our knowledge, this is the first study to assess the safety of packaged rice sold in UAE market in terms of OTA content, and to determine the exposure levels to this toxin from the rice consumption. OTA levels in rice samples collected in UAE (0.29±0.08 μg/kg) were higher than in some studies, but lower than in others. For example, in Brazil, from year 2007 to 2009, OTA concentrations ranged from 0.20-0.24 μg/kg in processed rice and its sub-products that were collected from different regions in the country (Almeida et al., 2012). OTA was not detected in any of the rice samples that were collected and tested in Ecuador (Ortiz et al., 2013). In Portugal, OTA contamination ranged from 0.09 to 3.52 μg/kg in different types of rice, including white rice and brown rice, and in different grain sizes (long and medium). This OTA range was within the EU limits, which is less than 5 μg/kg (Pena et al., 2005).

Nevertheless, OTA levels in white and parboiled rice ranged from 0.05 to 0.17 ng/g in Czech Republic, where 96.7% of the samples were positive, but all were below the limit (toman et al., 2016). On other hand, OTA contamination in rice was higher than our findings in Morocco and Iran, where it ranged from 0.08 to 47 μg/kg and from 0.65 to 11.54μg/kg, respectively (Juan et al., 2008, Nazari et al., 2014). Also, in Vietnam, Spain and United Kingdom, high amounts of OTA were recorded with levels ranging from 21.3-26.2 μg/kg, 4.3-27.3 μg/kg, and 1.0-19.0 μg/kg, respectively (Scudamore et al., 1997, Trung et al., 2001, Gonzalez et al., 2006). In a recent study in Kenya, OTA ranged from 0-111 μg/kg in rice samples where OTA was detected in 30% of the samples and 6% of the samples had an OTA level above the limit of 5 μg/kg (Mutiga et al., 2021). Finally, the moisture levels of the rice grains in our samples were below the maximum level of 14% of the sample weight, which can explain the relatively low levels of OTA compared to other studies.

Our findings showed that brown rice had a higher level of OTA (0.2 μg/kg) compared to white and parboiled rice (0.15 μg/kg), but this association was not

significant (p= 0.346). This can be explained by the presence of bran in the brown rice. These findings are consistent with other studies where a Canadian survey from 2018 to 2019 found that the only sample that has elevated OTA levels (11 μg/kg), which is above the Canadian limits of 3 μg/kg, was brown rice sample (Government of Canada, 2020). Also, in a Brazilian study, the average OTA level was highest in the rice bran (11.63 μg/kg) compared to rice, rice husk and broken rice where OTA levels were 1.78 μg/kg, 3.91 and 1.02 μg/kg, respectively (Almeida et al., 2012). Also, our study investigated the seasonal effect on OTA presence in rice. The climate in UAE is hot, humid and dry from April to September (IPS international, 2021). Warm and humid season can enhance the production of OTA when compared to cold seasons (Atumo, 2020). We found that rice packed in spring/summer (March to September), or fall/winter (September to March) did not show any significant difference in terms of OTA (p=0.62). This can be explained by the different packing countries that are located in different geographical areas; therefore, they do differ in weather conditions as OTA production is associated with the dry and humid season. The results, however, were consistent with moisture content percentages of all rice grains in our sample, which were below the maximum level of 14%. These findings could be justified by the adequate humidity and temperature management by the packing facilities. These outcomes are consistent with a study done in Lebanon to assess OTA levels in baby formulas where no significant differences in OTA levels collected in the fall/winter and spring/summer seasons were found (Elaridi et al., 2019). On the other hand, in Turkey, the highest OTA level in the samples of rice was found in winter, with an average of 1.11 μg/kg and significantly higher than in summer ($p<0.05$). These may be related to the highest relative humidity values (Buyukunal et al., 2010). Also, a study done in Vietnam found that rice samples taken during the dry season had a higher detection ratio, and OTA average was more than samples collected during the rainy season (Nguyen et al., 2007). Regardless of seasons, the broad temperature range of *Aspergillus* and *Penicillium* species cause OTA production to take place at any time all year round (Agriopoulou et al., 2020). Moreover, rice is cultivated in a setting that encourages fungus growth where its environmental conditions promote OTA growth and contamination. As a result,

contamination begins in the field (Sales &Yoshizawa, 2005). Then, under improper storage conditions, rice will be the perfect substrate for mycotoxin-producing fungi as *Aspergillus* and *Penicillium* (Toman et al., 2016). During storage, the ability of fungi to attack the rice grains and produce OTA is directly related to the water activity in the rice and temperature of the storage area. For that, when drying the rice, the aim is less than 14% moisture to minimize fungal growth and colonization upon storing (Mutiga et al., 2021). However, mycotoxin contamination in food products was not a food safety concern in Europe, but due to the present climate fluctuations and trends, the case has changed (Battilani et al., 2016).

Moreover, we studied the relation between the country of origin of rice and the level of OTA in the samples. Rice from developing countries of origin (India, Pakistan, Thailand, China) showed higher OTA contamination level compared to the developed countries (USA, Italy). Although there was a difference, it was not significant. These findings can be explained by the acceptable levels of moisture content found in all the rice samples. Mycotoxin concern is often more alarming in developing countries, especially in Asia, where the climatic conditions and the agricultural and storage techniques are favorable to the growth of fungus and generating toxins (Ali, 2017).

Furthermore, no significant association was found between rice packed in UAE and rice packed in other countries ($p=0.973$). These outcomes are also supported by the allowable moisture content values observed in all rice grains examined in our study. In the present study, OTA in rice collected from facilities having a food safety management system (FSMS), such as ISO22000, HACCP and FSSC22000, was lower than the levels in rice collected from facilities with no FSMS. This relation was significant ($p=0.04$). Some of the brands had missing information regarding the FSMS. For this reason and to avoid making assumptions, we categorized them in the "absence/information not available" class. The absence of data on FSMS does not exclude the chance of an unillustrated FSMS. It is important to mention that it is essential to recognize the difficulties that developing countries and rising economies cope with in reaching food safety regulations and systems (Trienekens & Zuurbier, 2008). In the UAE the government has a dedicated role which was an indispensable

driving force to encourage the implementation of a HACCP based food control systems in food industries (Al-Kandari and Jukes, 2011). Also, Dubai municipality has integrated the Food Watch platform in the FSMS of the industries. It facilitates data exchange between authorities, food businesses, service providers and consumers (Dubai Municipality, 2021). In addition to that, local order No:11, 2003 from Dubai Municipality as well, demands all food establishments to implement and maintain a risk-based food safety management system (Dubai Municipality, 2021). However, OTA contamination can occur in rice throughout the growing process before being shipped to other countries, requiring stricter FSMS conditions. Thus, from field to consumer, it is crucial to establish an integrated system based on the Hazard Analysis and Critical Control Point (HACCP) approach to control OTA and ensuring that it does not exceed the limits set by the legislation (Trienekens & Zuurbier, 2008; Ferre, 2016).

Furthermore, we found that long grain rice exhibited a greater OTA concentration than short or medium rice grains, but this difference was not significant ($p=0.175$). This higher level of OTA in long grain rice could be due to the higher surface area of this type of rice which might attract more molds, and thus OTA production. The Codex Alimentarius Commission Committee classified rice according to its length-to width ratio, where long grain has a ratio > 3:1, medium grain has ratio between 2:1 to 3:0, and short grain has a ratio <2:0 (Juliano, 1993). According to IRRI, the size of extra-long rice grains is >7.50 mm, long rice grains is between 6.61 to 7.50 mm, medium rice grain is between 5.51 to 6.60 mm, and short rice grains is <5.50 mm (IRRI, 2010).

Most mycotoxins are chemically stable; thus, OTA tends to survive the elimination of host fungi such as cooking in boiling water, baking bread, or long-time storage at proper conditions. Therefore, the absence of visible fungi mold in grains is not an indicator of being free from the contamination (Ha, 2015).

When assessing the effect of the time between packing and purchasing of rice, OTA levels was higher as this time increases. The highest OTA levels were in rice samples purchased after 30 weeks and above after packing (0.21 µg/kg), while the lowest OTA levels were in rice samples purchased between 1 to 9 weeks after

packing (0.03 µg/kg). This relation was significant in UAE (p=0.037). This can be due to poor barrier properties in the packaging process of some rice brands, as well as the poor storage conditions of rice in some retailers, which would increase environmental influence that affect the quality of rice. Also, it can be explained by the fact that most mycotoxins are chemically stable, thus OTA tends to survive the eradication of host fungi such as long-term storage. Therefore, the absence of seen fungi mold in rice is not a sign of being free from the contamination (Ha, 2015). On the other hand, airtight storage bags effectively prevent the spread of fungi to non-infected grains and block the influences of external humidity changes (Lane & Woloshuk, 2017), which explains the low OTA contamination even after 30 weeks of packing. Moreover, a study has shown that the first few weeks of using airtight bags caused the oxygen concentration to drop to approximately zero at grain moistures of 18 and 20% moisture content (Tubbs et al., 2017).

Our results showed a significant difference (p= 0.001) between the brands of both collections and OTA levels. This difference could be explained by the inconsistency of the manufacturing practices at both the processing and packing sites, which increase the vulnerability of OTA contamination in rice. Furthermore, improper storage conditions as temperature and humidity, and keeping rice grains with fungal contamination symptoms can lead to an escalated contamination level in these grains (Ferre, 2016). In another study in Africa, it was found that rice samples with inappropriate storage had a high likelihood of OTA growth (Tang et al., 2019). Moreover, when OTA producing fungi as *Aspergillus* and *Penicillium* spp. are assumed to be present, little can be done to prevent further contamination of fungi, and they can thrive over a broad temperature range of 10–40 °C with an optimum temperature ranging from 25–35 °C in the storage process. Thus, the only solution is to discard or get rid of the rice grains (Mahuku et al., 2019).

The tolerable daily intake for OTA is not counted as a safety factor because the intake of this toxin must be kept as low as possible. Accordingly, the Provisional Tolerable Daily Intake (PTDI) established by the Scientific Committee of Food (SCF) of the European Union was set at 5ng/kg body weight/ day (Toman et al., 2016). Based on the average OTA levels in our sample that was 0.29 µg/kg, the

anticipated daily OTA exposure can be determined. The expected daily OTA exposure in UAE is calculated based on the average body weights of 76 kg and the average consumption of rice of 56.1 kg/capita/year, which is equivalent to 153.7 g/capita/day. As a general rule, 1 cup of uncooked rice is equivalent to approximately 3 cups of cooked rice (University of Nebraska-Lincoln, 2021). Thus, 153.7 g of cooked rice is equivalent to 51.2 g dry rice/capita/day. For that, the expected daily OTA exposure is 0.20 ng/kg body weight/day, which is much lower than PTDI in EU

To our knowledge, no study has evaluated the exposure to OTA from rice consumption in UAE. Moreover, dietary OTA exposure from the rice in our sample was almost similar to that studied in Spain which was 0.17 ng/kg body weight/day (González et al., 2006), but lower than that studied in Czech Republic, Pakistan, Belgium and Iran which was 2.28, 4.2, 0.305 and 0.62 ng/kg body weight/day, respectively (Rahimi, 2014; Toman et al., 2016; Iqbal et al., 2016; Meerpoel et al., 2021). Also, our finding was higher than the dietary OTA exposure from the rice in Turkey and Italy which was 0.02 and 0.04 ng/kg body weight/day, respectively (Miraglia and Brera, 2007; Golge & Kabak, 2016). Although the results from various studies are valuable references, the comparisons should be carefully done since 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. Although the determined average daily exposure to OTA in our study is not remarkably high, it is crucial to keep the levels as low as possible to avoid the OTA harmful effects associated with increased rice consumption in UAE.

The strengths in our study include the fact that it was the first in UAE to assess the safety of packaged rice sold in the country in terms of OTA content, and to determine OTA exposure levels from rice consumption. In addition, we analyzed packed rice, as the majority of UAE citizens buy packed rice.

Concerning the limitations of our study, the results contained some outliers. This could be attributed to a 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 budgetary constraints, we had to conduct the analysis in duplicates. Furthermore, many samples from different brands had no FSMS information. We were unable to these food companies and manufacturer to obtain a clear response because the majority of them were unwilling to cooperate, which is why these samples were grouped in the "absence/no information available" category. Furthermore, 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 Seven

Conclusion

In conclusion, rice is one of the world's most widely consumed foods. Rice being contaminated with OTA is common in many countries. Our research found that rice in UAE is within the EU limits for OTA; thus, its consumption does not imply a significant health risk. However, surveillance strategies and monitoring procedures should be carried out regularly to ensure that rice is within the limits when it comes to OTA and other contaminants. On the consumer level, it is recommended to purchase packaged rice brands with food safety management system certification from recognized retailers and store the rice appropriately in the household to minimize the risk of OTA contamination. Future research should analyze the level of OTA in unpacked rice sold in various areas and food markets in UAE to obtain a general understanding of the quality of rice purchased and consumed. Also, upcoming research should look at the *aw* of the grains, which can be an indicator to OTA presence. Finally, ELISA results are recommended to be validated against the standard gold method of HPLC.

Chapter Eight

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