A multidisciplinary investigation into arsenic contamination in rice: rice preparation, arsenic knowledge and risk perception.

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List of Abbreviations

Arsenic (As)

Arsenobetaine (AB)

Arsenocholine (AC)

As (+3 oxidation state) methyltransferase (AS3MT)

Atomic absorption spectroscopy (AAS)

Atomic fluorescence spectroscopy (AFS)

Certified Reference Material (CRM)

Chronic Kidney Disease of unknown origin (CKDu)

Dimethylarsonic acid (DMA)

European Food Safety Authority (EFSA)

Expenditure and Food Survey (EFS)

Flow injection hydride generation atomic absorption spectrometry (FI-HG-AAS)

Flame atomic absorbtion spectroscopy (FAAS)

Gamma-ray spectrometer (GRS)

High resolution inductively coupled plasma mass spectrometry (HR-ICP-MS)

Hydride generation atomic fluorescence spectrometry (HG-AFS)

Inductively coupled plasma atomic emission spectroscopy (ICP-AES)

Inductively coupled plasma mass spectrometry (ICP-MS)

Inductively coupled plasma dynamic reaction cell mass spectrometry (ICP-DRC-MS)

The Food and Agriculture Organization of the United Nations (FAO)

Inorganic As (iAs)

Limit of detection (LOD)

Limit of quantification (LOQ)

Joint FAO/WHO Expert Committee on Food Additives (JECFA)

National Health and Nutrition Examination Survey (NHANES)

National Diet and Nutritional Survey (NDNS)

monomethylarsonic acid (MMA)

Recommended Daily Intake (RDI)

S-adenosylhomocysteine (SAH)

Trimethlarsine oxide (TMAO)

Tetramethylarsonium ion (TeMA)

UNFAO (United Nations Food and Agriculture Organisation)

Abstract

Rice is a popular staple consumed by approximately 3 billion of the world's population. It is a source of essential nutrients, carbohydrates, protein, vitamins and fibre. However, despite its high nutritional content, rice is the second most important route after water, for human exposure to arsenic (As) through the diet. Hence, awareness of As contamination in rice and use of mitigation techniques to reduce the As content of rice are vital.

The Overall aim of this thesis was to determine the As contamination levels in cooked rice considering different cooking methods used across the world and any effect of such cooking techniques on essential elements present in rice. Since consumption of rice and rice-based products are on the rise in the UK, one of our goals were to determine the awareness and risk perception of As exposure from rice intake in a UK population using a questionnaire based survey. Apart from rice, presence of As in other grains were also summarised using a systematic literature review.

The effect of cooking techniques on both As and essential elements showed a decrease in As of 4.5%, 30%, and 44% after using rice-to-water ratios of 1:3, 1:6 ($p = 0.004$), and 1:10 (parboiling; $p < 0.0001$) respectively. Similarly, increase in cooking water caused a decrease in essential elements, with the most decrease observed in potassium (K) (50%) and the least in copper (Cu) (0.2%) in comparison to the other elements. A further laboratory-based study investigated the As content of Sri Lankan rice and its association with CKDu. Results obtained were compared to existing literature and although it was established that rice from CKDu endemic areas might contain As, further investigation on the ecological risk of CKDu from As in rice is required. Results from the questionnaire survey revealed that general knowledge of As amongst the White British and ethnic minority groups was high. However, very few participants were aware of As contamination in rice. Prior knowledge of As in rice did not always result in the use of recommended practices. In comparison to consumers from the ethnic minority groups, the White British were more favourably inclined to reduce the amount and frequency of rice consumed, and consider food options other than rice. Thus, suggesting that the other ethnicities have low to no risk perception of As exposure through rice consumption whilst the White British may perceive risk of exposure to As from rice. Furthermore, results obtained from the survey revealed that apart from rice, other popular grains consumed include wheat, maize/corn and oats. This information formed the basis of the systematic literature review in chapter 7 and the results obtained showed that As contamination was higher (above 0.5 mg/kg limit for China and 1 mg/kg for Australia and New Zealand) in maize and millet in comparison to the other cereal grains. Results from this research could help rice eating communities to choose the best practice for rice preparation and consumption. Additionally, survey data provide unique information on dietary habits of ethnic minority groups, essential for dietitians and health professionals.

Chapter 1.

Introduction

1.1. Arsenic in rice

Arsenic (As) contamination is an international environmental issue (Brammer *et al*., 2008). Ground water As contamination in the South-East Asian countries, especially in Bengal delta has been described by WHO as the largest mass poisoning in history (Meharg, 2005). In Bangladesh alone, around 30 million people are exposed to As contamination in ground water. Due to its cumulative nature, As tends to concentrate in the rice grains, which makes rice an important source of As exposure (Melkonian *et al*., 2013). In South and South-East Asia, high As concentrations are present in some rice varieties due to the use of contaminated water during irrigation (Sengupta *et al*., 2006). Rice is affected the most because As is freely available to the plant roots in the wet soil conditions in which it is grown (Brammer *et al*., 2008).

In Europe and America where ground water As contamination is not a problem, apart from sea food, rice is another source of As exposure (Sengupta *et al*., 2006). Meharg *et al*. (2006) states that rice is a dominant contributor of inorganic As in the diet and according to Hojsak *et al*. (2015) the As concentration in rice is higher than that present in wheat and barley. The flooded conditions in which rice is grown and its ability to absorb As from the environment makes rice the most As contaminated cereal compared to other crops (FSA, 2015).

In recent years, there has been concern on the levels of As in rice and rice based products. This has led to discussions between regulatory bodies on how to deal with the problem of As contamination in this grain. As a result of these discussions, the Joint FAO-WHO Codex Alimentarius Commission has set a limit of 0.2 mg/kg of inorganic As for polished rice (Winter *et al*. 2015).

1.1.1. Improved cooking method as a form of arsenic mitigation in rice

Certain cooking methods can reduce the concentration of As in rice and they are considered as an immediate solution in lowering the dietary exposure to As. The most recommended method involves rinsing and cooking rice in excess water which is then discarded (Raab *et al*., 2009; Carey *et al*., 2015) compared to cooking rice to dryness with no water retained after cooking (Sengupta *et al*., 2006). The former is a traditional method mainly practiced in

Southeast Asia (especially in rural villages), whereas the latter is mostly practiced in the west. Sengupta *et al*. (2006) observed that cooking unwashed rice in the 1:2 ratio retained around 99.8% of As. On the other hand, Mihucz *et al*. (2007) carried out a study where Hungarian and Chinese rice were cooked using excess water (1:6 rice to water) and the results revealed a 39% and 60% decrease in As respectively. Most recently, Carey *et al*. (2015) devised a new technique of cooking rice in an apparatus that constantly condenses steam to produce a fresh supply of distilled hot water. This technique was successful in removing 59% of inorganic As. Additionally, Carey *et al*. (2015) also used percolation; a method which involved cooking rice in a coffee making device and this caused a 69% decrease in inorganic As.

1.1.2. Micronutrients in rice

Micronutrients are important for the correct functioning of the body and lack of or any imbalances are associated with disease aetiology. Conditions occurring from micronutrient deficiencies affect over 2 billion people worldwide (Harrison, 2011). Micronutrient deficiency of vitamin A, iron and iodine are a problem of public health importance in a number of countries, including India and Bangladesh (Kodish *et al*., 2011; Sivakumar, 2001). Damms-Machado *et al*. (2012) states that insufficient intake of essential micronutrients can have an effect on our everyday activities, behaviour, physical, intellectual and emotional state.

Rice is a staple for most Asian countries and it contains a variety of nutrients including proteins, carbohydrates and some essential elements. In poor Asian communities, vegetables are the most popular accompaniments to rice because they cannot afford or do not have access to other types of food, for example meat and fish from which they can obtain additional nutrients. This factor in addition to cooking rice in excess water, which results in reduction of micronutrients, increases the risk of micronutrient deficiency amongst the populations (Wieringa *et al*., 2014).

1.2. Diet of ethnic minority groups

The Economic and Social Research Council (ESRC) defines ethnic group as ‘people of the same race or nationality with a long shared history and a distinct culture’. One of the most important factors that govern the character of a particular ethnic group is their traditional diet. Although dietary acculturation amongst immigrants is common, food plays a major role in strengthening ethnic identity, preserving cultural traditions and easing homesickness (Azar *et al.*, 2013). Hence, traditional food is important in ethnic minority communities.

According to Stockley (2009), the dietary composition for ethnic minority groups can be influenced by their religious beliefs. In addition, the region of their origin plays an important role in determining their diet. For example, the staple food for most South Asian groups is rice and wheat. Indeed, the National Health and Nutrition Examination Survey (NHANES) carried out between 2005 and 2010 in the US acknowledges that ethnicity or race is an influencing factor when it comes to rice consumption (Nicklas *et al.*, 2014). FAO (2004) further suggests that properties such as taste, colour, texture and stickiness are important in the choice of rice for different cultures and regions of the world. For example, sticky rice is mostly preferred in Taiwan, Thailand, China, Japan and Korea where as dry rice is mainly consumed in South Asia and Middle East.

1.2.1. Rice consumption amongst ethnic minorities in the UK

The Expenditure and Food Survey (EFS) and the National Diet and Nutritional Survey (NDNS) provide data on the rate of rice purchase/ consumption in the UK. These surveys have shown that the largest rice-consuming group is the Asian-Bangladeshi with approximately 251g/d per capita, thirty times more than an average White Briton (Meharg, 2006). This is in agreement with a review carried out by Leung and Stanner (2011) which revealed that South Asians, in particular Bangladeshi's consumed rice the most in comparison to other ethnicities.

1.3. Aims and objectives

This research aims to address a knowledge gap in the area of rice cooking technique as a short term As mitigation technique and to also determine the awareness and risk perception of As exposure from rice intake in a UK population, using a questionnaire based survey.

A multidisciplinary approach encompassing cooking experiments, a questionnaire survey and systematic literature review was applied to address the following objectives:

- Quantify total As in UK, Sri Lanka, Myanmar and Nigerian rice varieties and determine the effect of traditional and conventional rice cooking methods on As and essential elements in rice **(chapter 4 and 5)**.

- Identify risk perception of exposure to As from rice intake amongst different ethnic groups in Manchester, UK and to explore whether knowledge about As contamination from rice has an influence on rice consumption and rice preparation practices **(chapter 6)**.
- Conduct a systematic literature review on As content of some popular cereal grains **(chapter 7).**

Chapter 2

Literature review

2.1. Arsenic and arsenic species

Arsenic (As) is the 20th most abundant element in the earth's crust. It is found in rock, soil, water, and air and exists in two forms, namely; organic and inorganic (Signes-Pastor *et al*., 2016; Rintala *et al*., 2014). Arsenic is a toxic element (Batista *et al*., 2011) and according to Tripathi *et al*. (2017) it can neither be removed nor destroyed from the environment, rather it can be transformed from toxic to less/non-toxic forms. The level of toxicity of As is dependent on its form, with inorganic As (iAs), which exists as arsenite (As(III)) and arsenate (As(V)) being more toxic than organic As (Zhao *et al*., 2010; Tripathi *et al*., 2017). Furthermore, As(V) is mainly found in oxidising conditions whilst As(III) predominates under reducing conditions (Nielsen and Larsen, 2014). Organic As exists as monomethylarsonic acid (MMA), dimethylarsonic acid (DMA), trimethlarsine oxide (TMAO), tetramethylarsonium ion (TeMA), arsenobetaine (AB), arsenocholine (AC), dimethylarsinylribosides, trimethylarsonioribosides, glycerylphosphorylarsenocholine and phosphatidylarsenocholine (Rintala *et al*., 2014; Davis *et al*., 2012; Zhao et a., 2010). There has been increasing concern due to the global prevalence of As exposure in humans over the past thirty years. (Chatterjee *et al*., 2010).

2.2. Exposure routes

The natural occurrence of As in the environment means that humans can be exposed to this metalloid through various routes including air (in form of As trioxide), soil, water and food (in form of arsenates and arsenites). Research has shown differences in the toxicity of inorganic As, with trivalent arsenites bearing more toxicity in comparison to pentavalent arsenates. For many years now, it has been established that inhalation contributes to the vast number of lung cancer cases involving workers employed in copper smelters and industries manufacturing arsenical pesticides (Smith *et al*., 2008). Amongst the As exposure pathways mentioned above, the most significant is through the ingestion of water and food (Tuzen *et al*., 2010; WHO, 2010; Ciminelli *et al*., 2017).

2.2.1. Arsenic in water and regulations

The occurrence of As contamination in groundwater is due to the release of As from aquifer sediments by biogeochemical weathering processes (Biswas *et al*., 2012; McCarty *et al*., 2011). Notably, the As found in water is virtually inorganic As, either as As(III) or As(V) (Hughes *et al*., 2011; Naujokas *et al*., 2013). Ground water in the regions shown in (**Fig. 2.1**) is accessed through tube wells which are sunk deep into the aquifers. Although this water is safe from bacterial contamination, the adverse effect is the high As concentration which the human population is exposed to through consumption (Meharg, 2006).

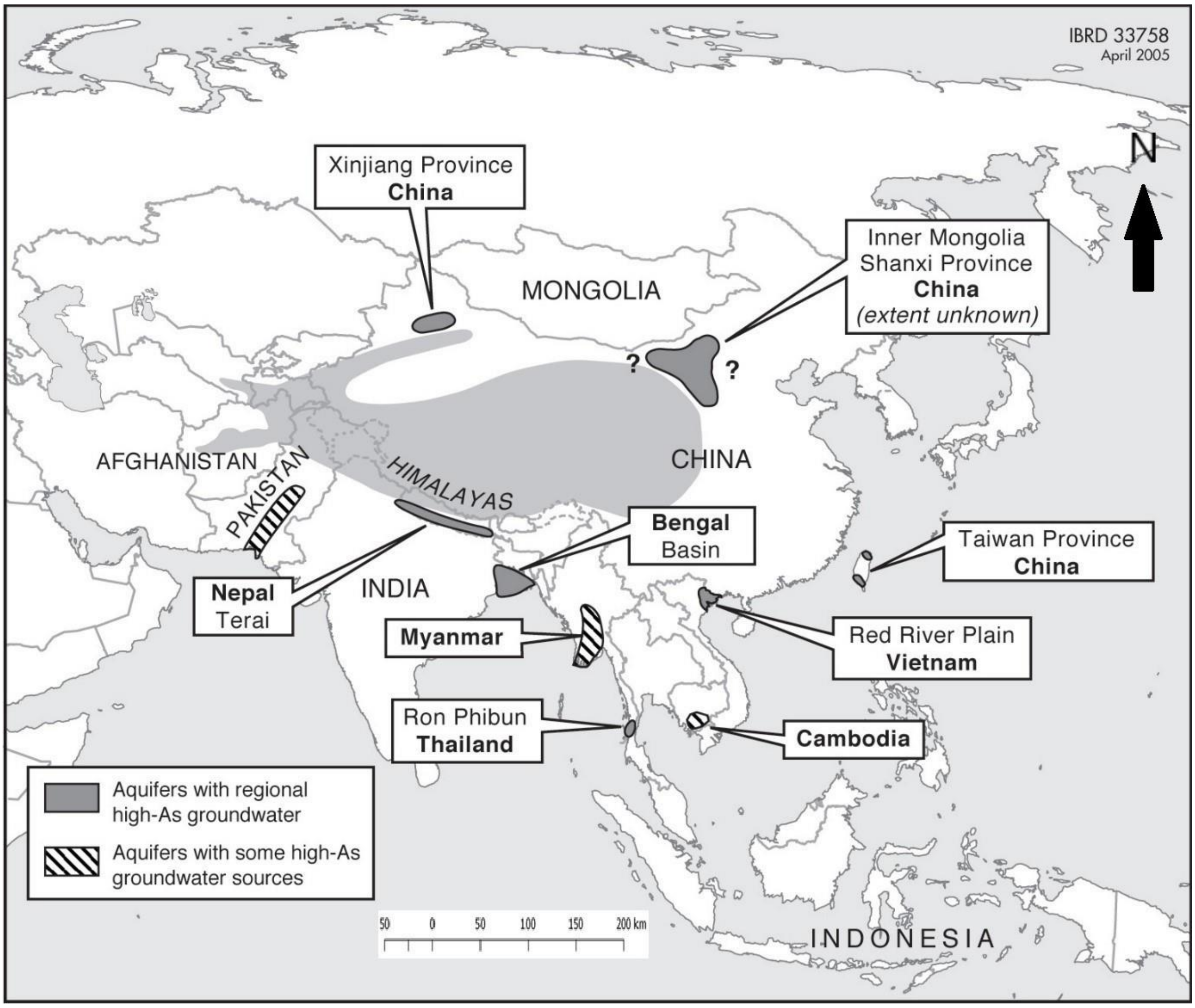

Figure 2.1. Regions in Asia affected by high ground water arsenic. Source: http://web.worldbank.org/WBSITE/EXTERNAL/COUNTRIES/SOUTHASIAEXT/0,,contentMDK:22392781~pagePK:146736~piPK:146830~theSitePK:223547,00.html

The contamination of ground and surface water by As is prevalent in many parts of the world, affecting more than 140 million people in approximately 70 countries but most especially in the Bengal Delta (Naujokas *et al*., 2013). More than 200 million people worldwide are affected by As in drinking water (Naujokas *et al*., 2013; Shankar *et al*., 2014), thus making it a global issue. Arsenic contaminated ground water has been linked to high levels of health risks amongst populations exposed to it (Sarker, 2010; Ali *et al*., 2010; Kumar *et al*., 2010; Yunus *et al*., 2016). The problem of exposure to inorganic As in drinking water was first identified in Taiwan, where some of population presented a range of skin and vascular lesions associated with As exposure (Drobna *et al*., 2009). In some countries like Bangladesh and India, efforts to reduce waterborne diseases and infant mortality caused by microbial contamination of surface water (main source of drinking water) led to As exposure through consumption of contaminated groundwater (Escamilla *et al*., 2011; Kile *et al*., 2016). Millions of tube wells connected to the groundwater were dug to provide safe and affordable drinking water, without the foreknowledge that the groundwater contained high levels of As (Zhao *et al*., 2010; Winston *et al*., 2013; Yunus *et al*., 2016). Unfortunately, this led to widespread As exposure in millions of people (Flanagan et al., 2012; Kile *et al*., 2016) thereby leading to As poisoning and the accompanying health risks. For example, Flanagan *et al*. (2012) states that about 35-77 million people in Bangladesh have been exposed to As contaminated drinking water, out of a total population of 140 million. According to Lokuge *et al*. (2004) it was observed that As caused 9,136 deaths per year and was responsible for 174,174 disability-adjusted life years lost per year, in populations exposed to concentrations above 50 µg/l. Furthermore, Latin American, European countries, USA, and Australia also have As present in the groundwater (Bundschuh *et al*., 2012; Sorg *et al*., 2014).

Due to the dangers of As toxicity, Sharma *et al*. (2013) and Santra *et al*. (2013) report that the WHO reduced the standard limit of As in drinking water from 50 to 10 µg/L in 1993. This set guideline for safe drinking water is duly implemented in the USA, Europe and China, hence drinking water in these areas is highly regulated (Zhu *et al*., 2008; WHO, 2011; Hite, 2013). However, in most As endemic areas like Bangladesh, the national guideline for As in drinking water is still set at 50 µg/L.

Detecting As in water non-scientifically is difficult because it is tasteless, odourless and colourless (Naujokas *et al*., 2013). Nevertheless, two main mitigation options which are the use of alternate water sources (such as piped water supply, rainwater, dug water wells and many others) and As removal technologies (use of filters, adsorption onto sorptive media, lime treatment and many others) have been employed to provide safe drinking water and help reduce human As exposure from drinking water in some endemic areas (Mondal *et al*., 2014; Sharma *et al*., 2013; Hossain *et al*., 2015).

2.2.2. Susceptibility of rice to arsenic

Arsenic contamination can occur as a result of natural causes or through anthropogenic sources. Activities such as mining, the use of pesticides and herbicides containing As and irrigation with As contaminated ground water have increased the As concentration in soil (Li *et al*., 2014). Due to this, humans are exposed to As through the soil-plant pathway. The growing conditions and the biology of rice make it the most efficient grain crop to accumulate As (Zhao *et al*., 2013). Rice grown in flooded conditions contains high As levels due to the increased bioavailability of As. In these conditions, As is converted to arsenite which strongly resembles the chemical properties of silicic acid, responsible for improving tolerance against biotic and abiotic stresses in plants (Ma, 2004). As a result, arsenite is able to fit into the silicic acid transporter proteins (Lsi1- a silicon influx transporter and Lsi2-a silicon efflux transporter) and hence it is taken up by the rice plant. In addition, arsenate, another inorganic form of As present in soil mimics phosphate, which is important for plant growth and maturity (Song *et al*., 2014). Furthermore, organic As in the form of monomethylarsonic acid (MMA) and dimethlyarsinic acid (DMA) are also taken up by the rice plant; however the rate of uptake is much lower than that of inorganic As. The reason for this could be the increase in hydrophobicity of the methylated As species (Zhao *et al*., 2013).

Drinking water is a major route of As exposure in areas with contaminated groundwater. However, in areas where As contamination in groundwater is not prevalent, for example Midnapur in West Bengal, cooked rice is the most dominant source of exposure to As (Mondal *et al*., 2010). Although As is present in high concentrations in foods like fish and sea foods, the inorganic form of this metalloid forms a small percentage of the overall As concentration. Nonetheless, the same cannot be said about rice, which can contain up to 90% of inorganic As (Hojsak *et al*., 2015). Additionally, cooking rice in As contaminated water increases the As content of cooked rice. It is also true that for countries not affected by drinking water As contamination, rice is the dominant contributor of inorganic As in the diet (Meharg, 2006). Inorganic As contributes about 20 to 90% of total As in rice (Xu *et al*., 2008). According to Hojsak *et al*. (2015) the As concentration in rice is higher than in other grains like wheat and barley. The flooded conditions in which it is grown and its ability to absorb As from the environment makes rice the most As contaminated cereal compared to other crops (FSA, 2015).

2.2.2.1. Arsenic concentration in rice of different origin and variety

Arsenic is present in all rice types. However, the concentration of total As in this grain is dependent on origin and variety. **Table 2.1** shows the As concentration in rice obtained from different countries (Cascio, 2011). As grains of rice take in As, they accumulate a disproportionate amount in their outer hulls, which are stripped off if the grains are refined into white rice (Bell, 2017). This is why brown rice, which has some nutritional benefits in comparison to white rice, has been found to contain more As (Consumer Reports, 2014). **Table 2.2** shows the total and inorganic As concentrations of some popular rice varieties.

Al-Rmalli et al. (2011) investigated the As content of rice varieties from Sylhet, Bangladesh and discovered that aromatic rice contained lower As content than non-aromatic rice. According to the study, consumption of aromatic rice reduces As intake by 70% and increases the intake of selenium and zinc by 40% in comparison to consuming non aromatic rice. Similarly, a study carried out by Sandhi et al. (2017) on Bangladeshi rice revealed that local aromatic rice (LAR) had a low As accumulation factor in comparison to high yielding varieties (HYV), thereby making LAR safer to consume in comparison to HYV.

Table 2.1. Total Arsenic concentration in rice sold in different parts of the world

Country	**Source of rice***	**Total As in rice (µg/kg)**	**Reference**
India (West Bengal)	G & P	130	(Mondal and Polya, 2008)
Bangladesh	G	143 (2-557)	(Rahman *et al.*, 2009)
Bangladesh		130 (30-300)	(Williams *et al.*, 2005)
China (polluted site)	G	490 (310-700)	(Xie and Huang, 1998)
Taiwan	G	200 (190-220)	(Schoof *et al.*, 1998)
USA		240 (110-340)	(Heltkemper *et al.*, 2001)
USA		260	(Williams *et al.*, 2005)
Europe		180 (130-220)	(Williams *et al.*, 2005)
Italy	G	(80-289)	(D'llio *et al.*, 2002)
Italy		220±10	(Williams *et al.*, 2005)
Spain	G &P	114±46	(Torres-Escribano *et al.*, 2008)
Spain		170±10	(Williams *et al.*, 2005)
Hungary	G	171.3±7.1, 139±6.1, 116±3.7	(Mihucz *et al.*, 2007)

*G = grown; P = purchased; mean (range); mean ± standard deviation.

Table 2.2. Total and inorganic arsenic concentrations of different rice varieties.

Product	Origin	Total As range (µg/kg)	Inorganic As range (µg/serving)
365 Everyday Value long grain brown	Info not provided by manufacturer	210 – 282	7.4 – 8.4
365 Everyday Value organic Indian basmati white	India	82.2 – 99.9	2.9 – 3.5
365 Everyday Value organic Thai jasmine white	Thailand	104 – 150	2.7 – 3.0
Archer Farms organic basmati	India	54.7 – 81.7	1.3 – 2.2
Archer Farms organic jasmine	Thailand	112 – 121	2.7 – 3.9
Cajun Country enriched long grain	LA	328 – 348	4.8 – 5.2
Cajun Country enriched popcorn long grain	LA	350 – 436	3.9 – 5.3
Canilla extra-long grain enriched	U.S.	198 – 431	3.2 – 7.2
Carolina enriched extra-long grain	AR, LA, TX	144 – 236	3.4 – 4.8
Carolina jasmine enriched Thai fragrant long grain	Thailand	119 – 159	3.0 – 3.2
Carolina whole grain brown	AR, LA, TX	277 – 318	6.4 – 8.7
Della basmati brown	AR	308 – 568	5.9 – 9.4
Della basmati white	AR	191 – 227	3.5 – 4.5
Uncle Ben's original enriched parboiled long grain	U.S.	220 – 246	5.9 – 6.3
Uncle Ben's whole grain brown	U.S.	209 – 285	5.7 – 6.7

AR – Arizona, LA – Los Angeles, TX – Texas, U.S. – United States, µg/kg – microgram per kilogram, As - arsenic. Table was adapted from Consumereports.org.

2.2.2.2. Arsenic in rice-based products

Apart from rice, rice-based foods are also an important source of As exposure through diet. Carbonell-Barrachina *et al*. (2012) investigated the As content of gluten free infant rice and infant cereals and discovered concentrations of 0.126 and 0.033 mg/kg respectively. A few years before that, Meharg *et al*. (2008) analysed total and inorganic As in UK baby rice and the highest levels recorded were 0.47 mg/kg and 0.16 mg/kg for total and inorganic As respectively. **Table 2.3** was adapted from Meharg (2006) and it presents data on total As levels in some rice products.

Table 2.3. Total As levels in rice products.

Product	Total As (mg/kg)
Liquids	
Vinegars	0.022
Wines	0.005
Japanese rice mirin	0.0320
Milk	0.0242
Baby foods	
Rice	0.183
Rice porridge	0.217
Rice cake	0.250

Mg/kg: milligram per kilogram. Adapted from Meharg (2006).

2.2.2.3. Regulations of arsenic in rice and rice products

The Codex Alimentarius Commission is an organisation that sets international food safety and quality standards to advocate safe and nutritious food for consumers worldwide. In July 2014, Codex adopted a maximum level of inorganic As in rice of 0.2 mg/kg, to mitigate the risks of exposure to As. The European Commission set maximum limits for As in rice and rice-based products, including rice destined for the production of infant food as follows (Commission Regulation, 2015);

a. 0.2 mg/kg for non-parboiled, white standard rice
b. 0.25 mg/kg for parboiled or husked rice
c. 0.3 mg/kg for rice waffles, crackers and other rice products
d. 0.1 mg/kg for infant food.

2.3. Arsenic metabolism and excretion

Upon ingestion, As is highly absorbed in the gastrointestinal tract. It is converted to a less toxic form in the liver hepatocytes through a process of methylation and the converted form is then excreted via urine (Drobna *et al.*, 2009). Research into the metabolism of inorganic As in mammals is based on the 19th century studies of As metabolism in microbes (Hughes *et al.*, 2011). The methylation of inorganic As involves oxidative and reductive processes which lead to the formation of mono, di and trimethylated arsenicals. Frederick Challenger proposed a pathway of As metabolism involving alternating oxidative and reductive steps (**Fig. 2.2.A**). Arsenate (As(V)) is converted into arsenite (AsIII)), monomethylarsonic acid (MMA(V)), monomethylarsonous acid (MMA(III)), dimethylarsinic acid (DMA(V)) and dimethylarsinous acid (DMA(III)). Reduction or oxidation interconvert As(III) and As(V) while methylation converts arsenite to MMA and DMA. The enzyme As (+3 oxidation state) methyltransferase (AS3MT) catalyses the transfer of a methyl group from S-adenosylmethionine (SAM) to trivalent arsenicals, producing monomethylated and dimethylated arsenicals which are excreted via urine. AS3MT is regulated by the concentrations of its substrates. Furthermore, inorganic As suppresses DMA production in a concentration dependent manner due to enzyme saturation (Peters, 2015). This results in the decrease of DMA:MMA ratio with increase in inorganic As. High concentrations of MMA also suppress DMA production due to substrate inhibition. In light of this, populations exposed to high levels of inorganic As are less efficient at completing the second methylation step, resulting in decreased DMA: MMA ratio (Song *et al.*, 2010 & Howe *et al.*, 2014). Another pathway suggested in As metabolism is known as the alternative pathway (**Fig. 2.2.B**). This involves the reduction of arsenate to arsenite by glutathione (GSH) or other endogenous reductants. Arsenite then undergoes an oxidative methylation, with SAM as the methyl donor, forming MMA^{V} and S-adenosylhomocysteine (SAH). MMA^{V} is reduced to MMA^{III} and then undergoes a subsequent oxidative methylation step to produce DMA^{V} and SAH.

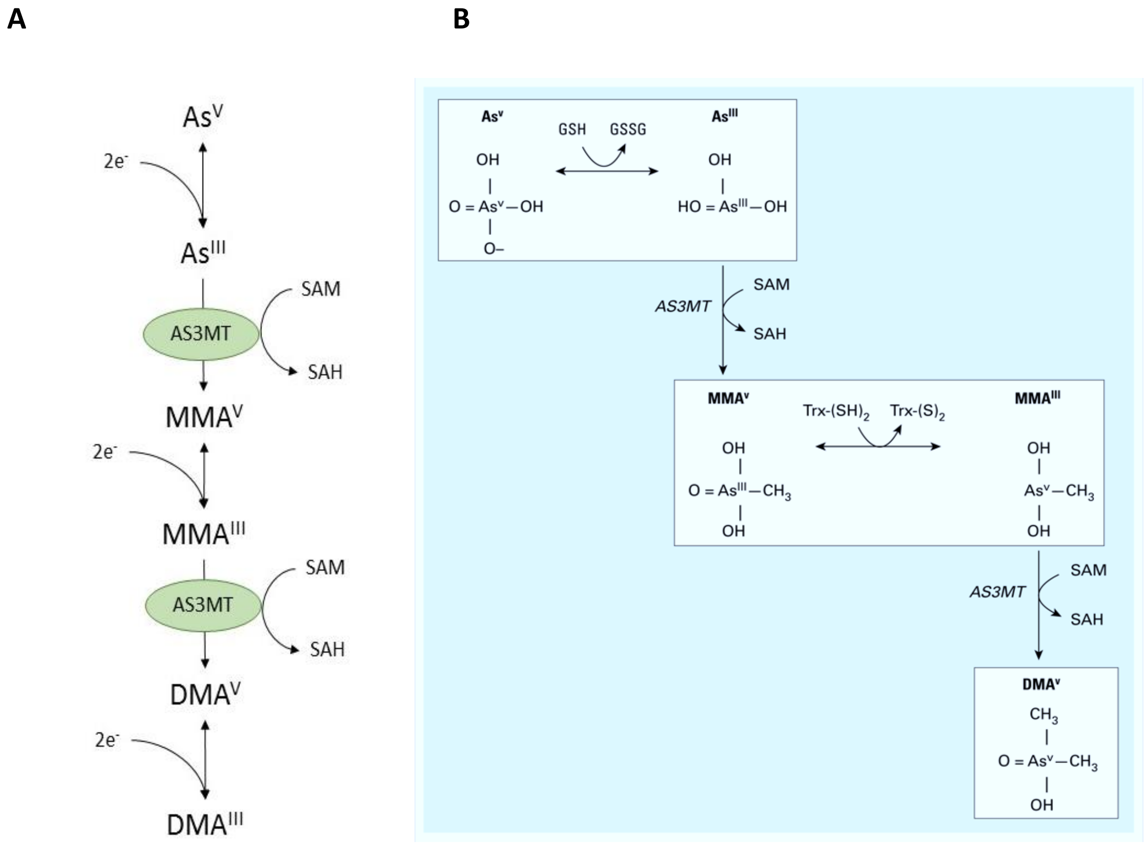

Figure 2.2. A. Challenger's pathway of arsenic metabolism (Peters, 2015) B. Alternative pathway of arsenic metabolism (Hall *et al*., 2009)

The presence of monomethylated and dimethylated arsenicals in urine was first reported by Crecelius in a study which involved a volunteer ingesting wine or water containing inorganic arsenite or inorganic arsenate (Crecelius, 1977). For many years, it was believed that the methylation of inorganic As was a detoxification process. However, more recently it has become apparent that the intermediates and products formed during this mechanism may be more reactive and toxic compared to inorganic As (Drobna *et al*., 2009). Therefore, in addition to detoxification, methylation can also be considered as a bioactivator of As.

2.3.1. Factors influencing the bioavailability, uptake and effects of arsenic

Research has shown that As toxicity is a precursor to multiple carcinogenic and non-carcinogenic conditions (IARC, 2012 and Lee *et al*., 2002). One of the most important factors to consider in susceptibility to As toxicity is inter-individual variations. Differences in individuals could affect As methylation capacities and metabolic patterns. In order to study individual susceptibility to As, urine can be used an indicator of toxicity. The table below indicates factors which are capable of affecting As methylation.

Table 2.4. Factors influencing arsenic methylation in individuals.

Factor	**Effects**
Diet	Poor diet and deficiency in certain nutrients is associated with high As toxicity, development of skin lesions and cancer (Pierce *et al*., 2011, Hsueh *et al*., 1997and Mitra *et al*., 2004).
Smoking	Cigarette smoking increases urinary As while chewing betel quid increases exposure to As (Al-Rmalli *et al*., 2011).
Drinking alcohol	Alcohol affects As methylation (Hopenhayn-Rich *et al*., 1996).
Ethnicity, gender and age	- According to Brima *et al*. (2006) ethnic origin plays a role in variations in methylation patterns of different ethnic groups in Leicester. - A study revealed that children poses a high As methylation rate when compared to adults (Concha *et al*., 1998). - Research by (Lindberg *et al.*, 2007; Lindberg *et al.*, 2008 and Steinmaus *et al.*, 2005) suggests that women have a higher methylation capacity compared to men due to estrogen, which acts as a cofactor in methylation. - Results from a study carried out on a population in Taiwan showed that As methylation in women and the young was higher in comparison with the men and elderly (Huang *et al*., 2009).
Genetic Polymorphism	- According to Antonelli *et al*. (2014) an individual's genotype affects the concentration of inorganic As metabolites in urine, hence this could also impact susceptibility to diseases caused by exposure to inorganic As. - Polymorphisms in AS3MT predicted As metabolism in two different populations from South America and Southeast Asia (Engstrom *et al*., 2011).

2.3.2. Effects of arsenic exposure

Arsenic is a ubiquitous, innate metalloid which has the ability to cause acute and chronic effects on several organ systems. It has been observed that acute inhalation of As fumes or dusts causes nausea, diarrhoea and abdominal pain. Likewise, acute oral exposure to As has been linked to effects on gastrointestinal tract, cardiovascular system, liver kidney and blood (EPA, 2012). A study carried out by Wassermann *et al*. (2004) on children in Bangladesh revealed that an increase in As exposure caused a decrease in the intellectual ability of these children, suggesting that As is also capable of affecting the central nervous system. Chronic exposure to As on the other hand leads to skin and mucous membrane irritation, skin lesions, hyperpigmentation, gangrene of the extremities and liver or kidney damage (ATSDR, 2007). Additionally, the International Agency for Research on Cancer (IARC) has classified inorganic As and its compounds as carcinogenic to humans, increasing the risk of skin, bladder, liver and lung cancer.

2.4. Global rice consumption

Rice plays a vital role as a staple for more than half of the world's population (Muraki *et al*, 2015). The genus *Oryza* comprise of about 25 species, distributed and cultivated in tropical and sub-tropical regions of Africa, Asia, South America and Northern Australia (FAO, 2015). Rice is consumed globally however; low-income countries have an increased demand for rice because it provides a cheap means of carbohydrates (FAO, 2015).

A steady increase in rice consumption per 1000 metric tonnes was observed worldwide between 2008 and March 2018 (**Fig. 2.3**).

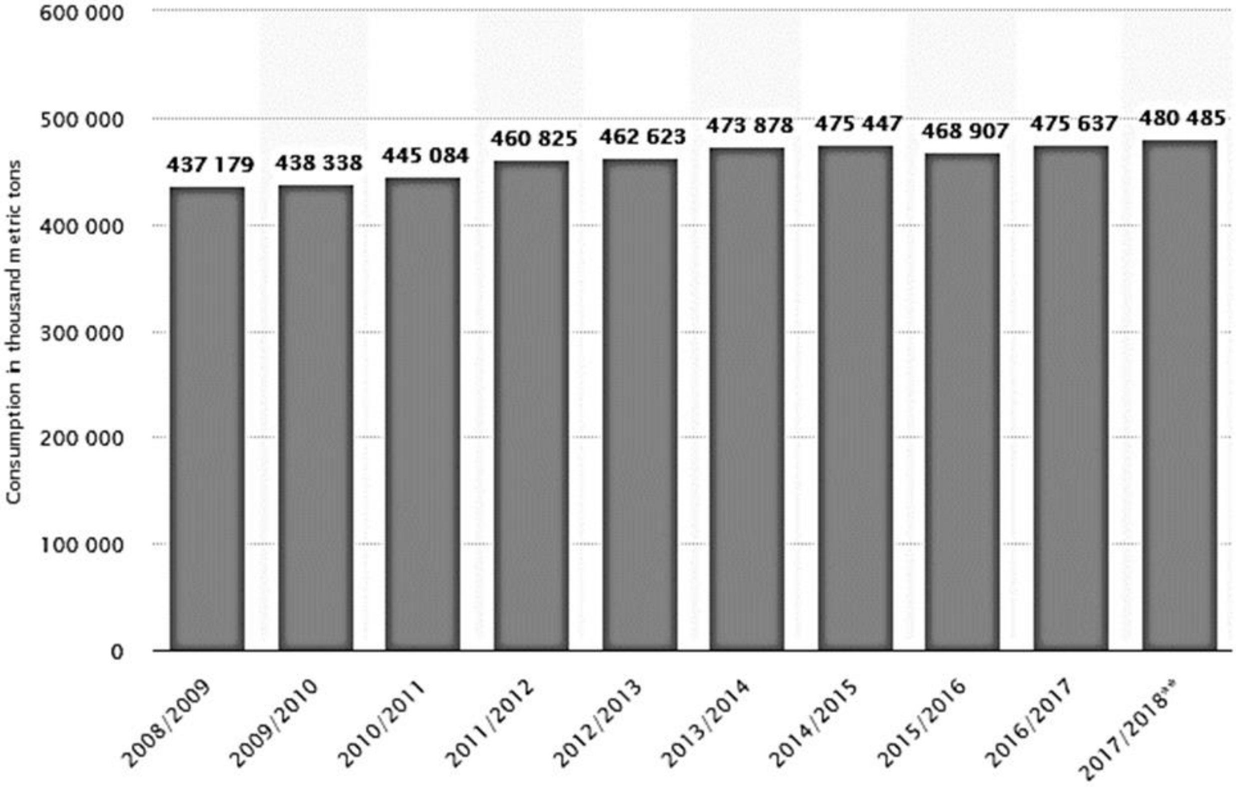

Figure 2.3. Data was sourced from the US Department of Agriculture; USDA Foreign Agricultural Service. Graph retrieved from https://www.statista.com/statistics/255977/total-global-rice-consumption/.

2.4.1. Asia

Rice production occurs almost worldwide but China, India and Bangladesh are the largest rice producing countries because the climate is best suited for cultivation (Akuzawa *et al*. 2002; Pinto *et al*., 2016). Rice is a staple for people in Southeast Asia and both cooked rice grains and processed rice flour are an important part of their diet (Akuzawa *et al*., 2002). It provides around 73% of the calorific intake of the population of Bengal region (Pal *et al*., 2009). Asia has also seen a rise in rice consumption per capita from 85 kilograms in the early 1960's to approximately 103 kilograms in the early 1990's due to the success of the Green Revolution (Mohanty, 2013). According to the United Nations, rice intake in this continent will continue to rise to cater for population increase and to compensate for the slow diversification away from rice to more high-value foods such as meat, dairy products, fruits, and vegetables, due to low income amongst the population (Maclean, 2013). Between 2012 and 2014, Sri Lanka saw an enormous rice consumption rate of 2.7 million tons per year (Mustard and Wright, 2013).

Apart from being consumed as a meal, rice has also been used in the production of other rice-based foods and drinks. For example, in Japan cooked and uncooked rice grains are the main ingredients in ‘sake brewing’- a process which converts the starch content of rice into sugars before being converted into alcohol (Teramoto *et al*., 1994).

2.4.2. Africa

The International Rice Research Institute (IRRI) reports that rice consumption per capita in sub-Saharan Africa has risen by more than 50% in the past two decades (Mohanty, 2013). Nigeria in particular has seen an increase in dietary rice consumption due to ease of accessibility and multiple ways in which it can be prepared (Seifarth, 2014). Total rice consumption in Nigeria grew by 6% per year between 2006 and 2010. In western, eastern and southern parts of Africa, the upsurge in rice consumption can be attributed to urbanisation, ease of preparation and the availability of better quality of rice imported to bridge the gap between regional supply and demand (Ricepedia, 2016). In addition, rapid population growth in Sub-Saharan Africa has also led to increase in rice demand (FAO, 2015).

2.4.3. Europe

In Europe, most of the rice production is located in Spain and Italy, which accounts for 80% of the overall European production (Pinto *et al*., 2016). Annual consumption per capita is around 3.5 to 5.5 kg milled rice in northern Europe and 6 to 18 kg in southern Europe (Maclean, 2013)

The National Diet and Nutrition Survey (NDNS) has provided data on food consumption and nutritional status in the UK since 1990 (Nelson *et al*, 2007). Survey carried out between 2008 and 2012 revealed that rice was among the cereals consumed by over 70% of the population and it contributes to the diet by providing important nutrients to the population (Schenker, 2012).

2.4.3.1. Rice consumption amongst Ethnic and Caucasian populations in the UK

Statistics have revealed that between 87 and 95 grams of rice was consumed per person per week in the UK between 2006 and 2013 (Statista, 2016). **Table 2.5** gives a breakdown in rice purchase of different ethnic groups. Rice purchase is considered to be consumption rate. The

Bangladeshi population is by far the largest rice consumer in the UK in comparison to other ethnicities. According to Cascio *et al*. (2010) Bangladeshis consume 30 times more rice than White Caucasians.

Table 2.5. Daily rice purchase per adult (g/d).

Group	Dried rice	Cooked rice	Take-away rice	Total
White				
White-British	4.4	0.8	3.0	8.2
Other white	6.5	0.7	2.7	9.9
Mixed				
White and black Caribbean	5.0	0	12.9	18.0
White and black African	39.3	0	0	39.3
White and Asian	17.8	0	0.8	18.6
Other mixed	12.9	0	2.5	15.4
Asian/Asian British				
Asian-Indian	25.5	0	2.5	28.0
Asian-Pakistani	29.1	0	0.5	29.6
Asian-Bangladeshi	250.6	0	0	250.6
Other Asian	59.7	0	1.7	61.4
Black/Black British				
Black Caribbean	33.5	0	3.0	36.5
Black African	31.6	0	1.1	32.5
Other black	43.5	3.4	1.4	48.3
Chinese and other				
Chinese	34.9	0	0.3	35.2
Other ethnic background	114.8	0	2.9	117.7

Rice purchase data from the 2005 Expenditure and Food Survey. Adapted from Meharg (2006). g/d – grams per day.

2.5. Importance of essential elements in rice

Rice is one of the most popular staple choice because it is high in starch, low in fat and cholesterol and easy to digest (Rice Association, 2016). **Table 2.6** shows the concentration of some essential elements in long-grain white and brown rice.

Table 2.6. Essential elements contained in cooked long-grain white and brown rice (per 100 g) and contribution to Recommended Daily Intake (RDI).

Element	**Long-Grain White Rice**		**Long-Grain Brown Rice**		**RDI for Adults**
	Amount (mg)	**RDI%**	**Amount (mg)**	**RDI%**	
Calcium	10	1	10	1	1000 – 1200 mg/d
Iron	1.2	7	0.42	2	8 – 11 mg/d (males) 8 - 18 mg/d (females)
Magnesium	12	3	43	11	400 – 420 mg/d (males) 310 – 320 mg/d (females)
Phosphorus	43	4	83	8	700 mg/d
Potassium	35	1	43	1	
Sodium	1	0	5	0	
Zinc	0.49	3	0.63	4	11 mg/d (males) 8-9 mg/d (females)
Copper	0.069	3	0.1	5	900 µg/d
Manganese	0.472	24	0.905	45	2.3 mg/d (males) 1.8 mg/d (females)
Selenium	7.5 mcg	11	9.8 mcg	14	55 µg/d

RDI refers to the recommended daily intake, mg – milligram and µg – microgram. Nutrient information from USDA. Adapted from: http://skipthepie.org/cereal-grains-and-pasta/rice-white-long-grain-regular-cooked/compared-to/rice-brown-long-grain-cooked/

2.6. Nutritional deficiencies

Micronutrient deficiency or ‘hidden hunger’ as described by Mohanty (2013) is a global public health problem that is commonly found in developing countries. Tulchinsky (2015) explains that not only can it lead to certain diseases, but it can also intensify the symptoms of some infections and chronic illnesses. According to Hotz *et al*. (2014) zinc deficiency in most common nutritional deficiency in Bangladesh, affecting around 57% of non-pregnant women and 45% of preschool children.

Harinarayan *et al*. (2004) carried out a study in India to investigate the dietary habits of rural and urban populations and how this was related to serum calcium, phosphorus and vitamin D. The results showed that the rural population had low dietary calcium intake, below the recommended daily allowances.

According to Lu *et al*. (2013) low concentrations of essential elements like zinc and iron in staple foods are the main cause of micronutrient malnutrition in developing countries. Another factor contributing to micronutrient deficiency is the presence of high levels of phytic acid in rice and vegetables. Phytic acid/myo-inositol hexaphosphate is highly negatively charged, causing it to form stable complexes (phytate) with mineral ions and thereby reducing their bioavailability in the gut (Lopez *et al*., 2002). Some of the nutrients inhibited by phytic acid are iron, zinc, calcium, manganese and magnesium (Herath *et al*., 2011).

Many believe that rickets and osteomalacia in countries that experience abundance in sunshine is a rare occurrence. However, this belief has been discounted by different studies. One such study was carried out by Harinarayan *et al*. (2004) who investigated the dietary habits of rural and urban populations in India and their relationship with serum calcium, phosphorus and vitamin D. The results showed that not only was their dietary calcium below the recommended daily intake but the participants were also insufficient in vitamin D. More interestingly, it was also observed that rural participants had high phytate levels due to the consumption of *eleusine coracana* also known as ragi/finger millet. As a result, calcium bioavailability in the gut was reduced. A similar study carried out by Karunaratne *et al*. (2008) examined the levels of zinc, iron and phytic acid in some popular foods consumed by Sri Lankan children in the rural areas. The study revealed that rice based meals with vegetable accompaniments were the most popular choice amongst the children and the high phytic acid to zinc ratio in these foods is a worrying factor in the bioavailabilty of essential elements.

Reliance on polished rice stripped of its nutrients, the lack of varied diet and rice cooking technique could all contribute to micronutrient deficiency which has severe ramifications in children, pregnant women and the elderly. This definitely calls for more research in the diets of populations in developing countries but most importantly, solutions are needed in order to tackle micronutrient deficiencies in these countries.

2.7. Rice preparation

Due to widespread consumption of rice, method of preparation and choice of cooked rice texture differ from one region to another. Das *et al*. (2006) highlighted the different preferences in some parts of the world; countries in the west enjoy long-grain, light, fluffy or slightly dry single rice grains with flavour and no hard core. Japanese consumers on the other hand prefer short-grain sticky rice whilst Indians like medium-grain, light, fluffy individual grains with flavour and a soft core.

Soaking of rice before cooking is a common practice that is done with the aim of achieving quick and uniform water absorption. This is of particular importance with brown rice as it is more resistant to water absorption in comparison to white rice (Han and Lim, 2009). In addition to improving the moisture content of rice, soaking also reduces the cooking time by causing quick heat transmission during cooking (Roy *et al*., 2011). The importance of rice preparation has been emphasised in the studies carried out by Ebuehi and Oyewole (2007) and Han and Lim (2009). These studies explored the effect of soaking and cooking on the physical characteristics, nutrient composition, sensory evaluation and digestive properties of different rice varieties and the results revealed that each of these characteristics are affected by cooking technique.

Rinsing is an important step in most cooking procedures as it washes off dirt and impurities. In the case of rice, not everyone agrees; an article in foodreference.com (2016) states that rinsing is not necessary in the modern kitchen because rice does not contain dirt or polishing additives.

Rice to water ratio is important in rice cooking. Optimal-water cooking involves using rice to water ratios of between 1:1.5 and 1:2.5 (Chakkaravarthi *et al*., 2008). Sengupta *et al*. (2006)

carried out a study in which three different cooking methods were explored; a traditional method commonly used in the Bengal delta involving a rinsing step and cooking rice in excess water (5-6 times the weight of rice) which is later discarded. The second technique involved rinsing and cooking rice in water twice the weight of the rice so that no water was left to discard. The last method, referred to as the contemporary technique comprised cooking unwashed rice in approximately twice the weight of the rice until no water was left to discard.

Roy *et al*. (2011) acknowledge that cooking technique has a great impact on the overall concentration of As in rice. Nonetheless, they observed that parboiled rice tends to contain more As in comparison to non-parboiled rice if As contaminated water is used in the parboiling and cooking processes. Therefore the risk of exposure can be reduced by consuming less parboiled and more non-parboiled rice in As endemic areas.

In addition, not only does preparation affect the physical and chemical properties of rice but it also influences the content of essential elements and toxic heavy metals that might be present in this staple.

2.7.1. Literature on the effect of cooking method on arsenic content of rice

Rinsing and modifying rice cooking water volume can affect the level of exposure to inorganic As. Raab *et al.* (2009), Sengupta *et al.* (2006), Rahman and Hasegawa *et al.* (2011) and Meharg and Zhao (2012) reported losses of between 28% and 60% of total and inorganic As in rice that underwent rinsing and cooking in water containing low As levels.

Table 2.7 gives a summary of 2 studies which investigated the effect of washing, using the 1:2.5, 1:6 and steaming methods on the As content of rice. Raab *et al*. (2009) observed a decrease of between 1 to 15.3% in total As after rinsing the different rice varieties in distilled water. In his study the most decrease in As was seen after cooking using the 1:6 technique in comparison to the 1:2.5 and steaming methods. Rahman on the other hand cooked rice from contaminated and uncontaminated areas using As contaminated cooking water. The results revealed a massive increase in the As content of different rice varieties cooked using the 1:2.5 method in comparison to the 1:6 technique; where the only increase of 16.7% was recorded in BRRI hybrid dhan1 rice.

Table 2.7. Percentage change in As concentration after different cooking techniques.

Rice variety	Raw – Washed (%)	Cooking Method			Reference
		Raw - 1:2.5 (%)	Raw - 1:6 (%)	Raw - Other (%)	
Basmati (Polished)	↓13.0	↓13.0	↓36.4	Steamed ↓24.7	Raab *et al.* (2009) Rice was cooked using double distilled water.
Basmati (Wholegrain)	↓15.3	↓9.2	↓45.0	↓9.2	
Long-grain (polished)	↓3.1	↑3.9	↓27.9	↓22.7	
Long-grain (Wholegrain)	↓1.0	↑3.2	↓30.3	↓10.8	
Italian *parboiled	↓3.8	0.0			
Long-grain (*Parboiled)	↓3.2	↓12.4			
BRRI dhan28 (non As contaminated area)		↑23.8	↓19.0		Rahman *et al.* (2006)
BRRI hybrid dhan1 (non As contaminated area)		↑33.3	↑16.7		
BRRI dhan28 (As contaminated area)		↑31.6	↓31.6		
BRRI hybrid dhan1 (As contaminated area)		↑58.0	↓36.2		

↓ Decrease, ↑ increase, % percentage. *Parboiled refers to rice which has undergone soaking, steaming and drying before it is polished.

Chapter 3

Optimisation of rice cooking method

3.1. Abstract

Optimisation of the rice cooking method was carried out to determine the best preparation, digestion and dilution factors for the analysis of As and micronutrients in rice. 0.2g of CRM was digested and diluted using the 2 and 4 dilution factors whilst 0.5g of CRM digested and diluted using the 2 and 4 dilution factors to determine the best method for analysis. According to the results obtained from ICPOES and ICPMS analysis, the best dilution factor was the 0.5g CRM and 2 dilution factor because the recovery of the majority of the elements was close to or just about 100% of the actual CRM values. From then on, this method was used in the analysis of rice samples. Rice was cooked in As free (0 mg/L) and contaminated water (0.01 mg/L, 0.05 mg/L and 0.10 mg/L) using two cooking methods (1:3 and 1:6). Cooking in As free water decreased the As concentration whilst cooking rice in As contaminated water increased the As retained in the cooked rice. These results are in line with literature on similar cooking studies that have been carried out. Cooking rice in excess water (1:6) caused a loss in Mg (67.1%), Ca (70.1%), K (89.5%), P (60.3%) and Fe (32.1%) in long-grain white rice. A loss in K (67.3%), P (19.2%) and Fe (0.99%) was also observed in long-grain brown rice, however there was an increase of 0.9% and 71.4% in Mg and Ca respectively.

3.2. Introduction

3.2.1. Rice preparation

Rice preparation which aims at reducing the concentration of inorganic As is seen as an immediate solution in lowering exposure to inorganic As through the diet. The most recommended method involves cooking rice in excess water which is later discarded (Raab et al., 2009) compared to cooking rice to dryness where no water is retained afterwards (Sengupta et al., 2006). Although research is being carried out on different cooking methods as a way of reducing As concentration in rice, the nutritional value of the end product (cooked rice) is neglected. The current study is aimed at establishing the effectiveness of two popular methods (1:3 and 1:6) of rice cooking on retaining important micronutrients in two rice varieties.

3.2.2. Micronutrients in rice

Micronutrients are important for the correct functioning of the body, lack of or any imbalances are associated with disease etiology. Conditions occurring from micronutrient deficiencies affect over 2 billion people worldwide (Harrison, 2011). Micronutrient malnutrition in vitamin A, iron and iodine are a problem of public health importance in a number of countries, including India and Bangladesh (Kodish *et al*., 2011 & Sivakumar *et al*., 2001). Damms-Machado *et al*. (2012) highlights that insufficient essential micronutrients have an effect on our everyday activities, behaviour, physical, intellectual and emotional state. Rice is a staple for most Asian countries. It is the main source of nutrients for the population because they cannot afford or do not have access to other types of food, for example meat and fish from which they can obtain additional nutrients.

3.3. Materials and methods

Rice Certified Reference Material (CRM) 1568b, purchased from the National Institute of Standards and Technology (NIST) was used to optimise the method for elemental analysis using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP OES) and In Inductively coupled plasma mass spectrometry (ICPMS). The purpose of this optimisation was to determine the best reagent volume, sample weight and dilution factor to be used in the analysis of heavy metals and essential elements in rice. Figure 3.1 summarises the conditions for the optimisation.

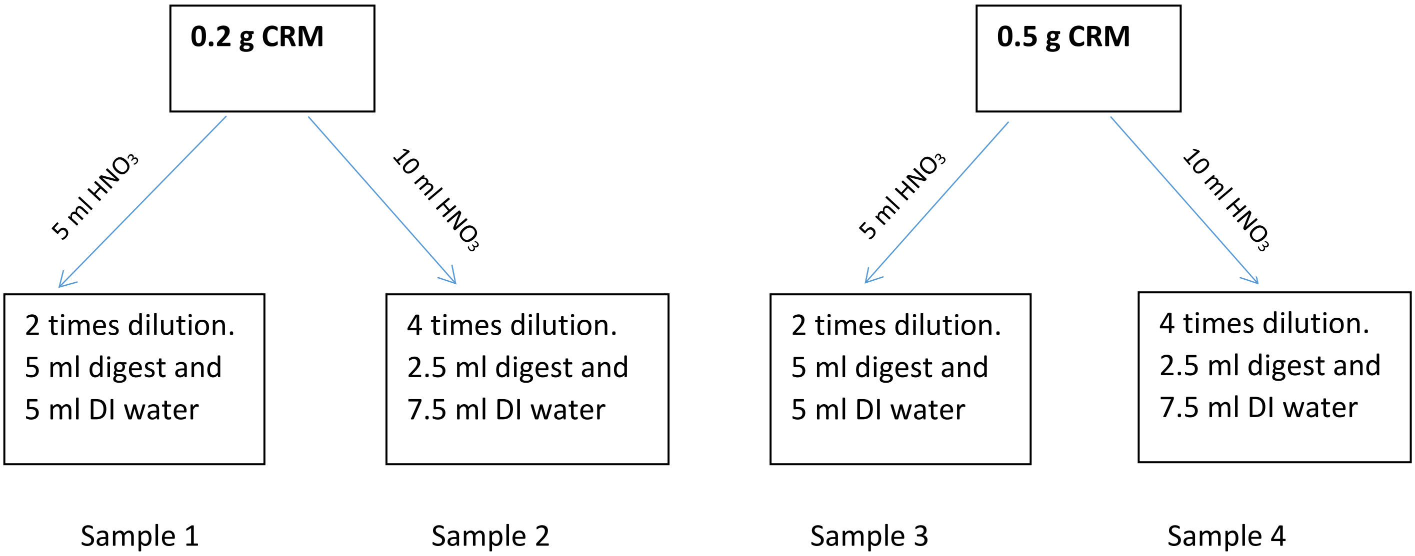

Figure 3.1. Conditions for protocol optimisation using rice CRM. HNO_3 – Nitric acid, CRM – Certified Reference Material and DI – Deionised.

3.3.1. Rice preparation

Tesco easy cook long grain rice (white) and Tesco easy cook brown rice where used in this experiment. According to the description on the packet, these rice samples originated from multiple countries. 10 g of each type of rice was weighed out into 4 individual beakers and rinsed with deionised water until the rinse water was clear. The rice was cooked in As concentrations of 0 mg/L (milligram per litre) (no As), 0.01 mg/L (WHO standard), 0.05 mg/L (Standard followed in South-East Asian countries including India) and 0.10 mg/L (highly contaminated water). In addition, rice was cooked in the ratios 1:6 (six times more water than rice); a traditional method of the Indian sub-continent, which is also found to be effective in As removal (Sengupta *et al*., 2006) and 1:3 (three times more water than rice); a contemporary method (Sengupta *et al*., 2006). Rice samples were cooked on hot plates at 385°C until soft consistency was achieved, after which the rice was left to cool down and the remaining water was discarded (only in the case of 1:6 method). The 1:3 method involved cooking the rice to dryness, therefore no water was left to discard. The rice was then dried in a 40°C oven for 72 hours before being transferred to a 110°C oven for 48 hours/until constant weight was attained. After the drying process, the rice was ground to powder using a mortar and pestle and stored in a desiccator until digestion.

3.3.2. Digestion

Rice digestion was done following the University of Manchester standard protocol. 0.5g rice powder was weighed using an analytical balance (PS-100) and transferred into already labelled microwave vessels. 5ml nitric acid was added to the rice powder and the mixture was left in a fume hood overnight before microwave digestion. The samples were accompanied by analytical blanks and certified reference material – NIST SRM 1568b rice flour. A MARS microwave system was used under the following conditions;

- 10 minutes to reach 170 ^{0}C then 20 minutes heating time at the same temperature
- Maximum power of 1600W

Total run time of 30 minutes and 40-45 minutes cooling time.

After digestion, 18Ω deionised water was added to the digests to make up a total volume of 50ml.

3.3.3. Analysis of rice samples

2ml of the prepared rice digests were added to ICP tubes containing 8ml of 18Ω deionised water to make up a final volume of 10ml. The Inductively Coupled Plasma Mass Spectrometer (ICP-MS) (Agilent 7500cx) and the Inductively coupled plasma atomic emission spectroscopy (ICP-AES) Perkin-Elmer Optima 5300 dual view were used for metal analysis. For the purpose of this study the elements of focus were As *(m/z 75),* Fe *(m/z 56)*, Ca *(317.933)*, Mg *(280.271)*, K *(766.490) and* P *(178.221).*

The methodology for this study was validated by successfully determining As, Mg, K, P and Fe in the reference material NIST SRM 1568b rice flour. Elements with values below detection limit were given the value of limit of detect (LOD)/2; As (0.01) and Fe (0.1).

Figure 3.2. Photos of researcher during ICP-OES analysis.

a. Photo of the researcher presenting samples to the ICP-OES instrument for analysis.

b. Researcher observing the results from the analysis.

3.4. Results

The best results (element concentrations) obtained after the optimisation were from sample 3 (Table 3.1) which comprised 0.5 g CRM, 5 ml nitric acid and 2 times dilution. In addition, the percentage recoveries for most of the elements were close or slightly above 100% (**Table 3.1**). Therefore, this protocol was used for the actual analysis in the present study.

Table 3.1: Results obtained from the optimisation of CRM.

	Element Concentration (mg/kg)						
Sample	**As**	**Cd**	**Ca**	**Fe**	**Mg**	**Mn**	**Zn**
1	0.740	4.822	117.403	7.409	315.290	12.335	15.177
2	-4.692	19.045	215.281	18.303	439.363	20.518	19.002
3	0.362	-1.258	106.525	7.436	549.070	19.492	17.990
4	0.430	0.216	142.603	9.461	601.121	19.899	19.906
Reference Values	0.285 ±0.014	0.0224 ±0.0013	118.4 ± 3.1	7.42 ± 0.44	559 ± 10	19.2 ± 1.8	19.42 ± 0.26
% Recovery	127.0	0.0	90.0	100.2	98.2	101.5	92.6

As- As, Cd- Cadmium, Ca- Calcium, Fe- Iron, Mg- Magnesium, Mn- Manganese and ZN- Zinc.

3.4.1. Quality assurance: Certified reference material recovery

Certified Reference Material (CRM) and percentage recovery values for the elements of interest are shown in the table below (**Table 3.2**). High CRM recovery values for the elements of interest were observed after analysis. This shows good analytical methods.

Table 3.2. CRM recovery (n = 3)

Element	CRM Value (mg/kg)	Recovery Value (mg/kg)	% Recovery
As	0.285 ± 0.014	0.267	93.7
Ca	118.4 ± 3.1	113.550	95.9
Fe	7.42 ± 0.44	6.170	83.2
Mg	559 ± 10	415.642	74.4
K	1282 ± 11	1099.816	85.8
P	1530 ± 40	1505.849	98.4

Replicates

Table 3.3. 0.01 mg/L arsenic water

Cooking Method	Rice Variety	Replicates (mg/kg)		% difference
		R1	R2	
1:6	white	0.004962	0.004979	0.34
	brown	0.004991	0.004865	2.52
1:3	white	0.004902	0.00499	1.76
	brown	0.00499	0.004739	5.03

Table 3.4. 0.10 mg/L arsenic water

Cooking Method	Rice Variety	Replicates (mg/kg)		% difference
		R1	R2	
1:6	white	0.600322	0.592116	1.37
	brown	0.476069	0.444385	6.66
1:3	white	0.927919	0.498572	46.27
	brown	0.450148	0.396069	12.01

The majority of the replicate values at the two As water concentrations are close to each other, thereby showing good precision (**Table 3.4**).

3.4.2. Arsenic concentration in raw and cooked rice

The results below (**Table 3.5**) show a higher total As concentration (hereafter referred to as As concentration since no speciation was done to estimate the inorganic and organic As forms) in raw brown rice compared to raw white rice. In addition, a decrease in As is seen upon cooking using both 1:3 and 1:6 cooking methods (91.6% and 91.8% in white rice and 93.5% in brown rice respectively). However, there was no difference in the As concentration in brown rice when cooked in 1:3 and 1:6 ratios.

Table 3.5. Arsenic concentration in raw vs rice cooked in As free water (n = 1)

Rice condition	**As Concentration (mg/kg)**	
	White long-grain rice	**Brown long-grain rice**
Raw	0.0600	0.0766
Cooked 1:3	0.0050	0.0050
Cooked 1:6	0.0049	0.0050

3.4.3. Effect of arsenic contaminated water on arsenic concentration in cooked rice

The relationship between As concentration in cooking water and As concentration in cooked rice was investigated (**Fig. 3.3**). At 0.01 mg/L As concentration (water), no significant change was observed in the As retained by the cooked rice in both the 1:3 and 1:6 cooking methods. At 0.05 mg/L however, the long-grain brown rice cooked using the 1:3 method retained more As compared to the rest. On the other hand, the long-grain white rice cooked using the 1:6 method retained the lowest As. Surprisingly, a decrease was seen at 0.10 mg/L in the As retained in long-grain brown rice cooked using the 1:3 method. Further investigation is required.

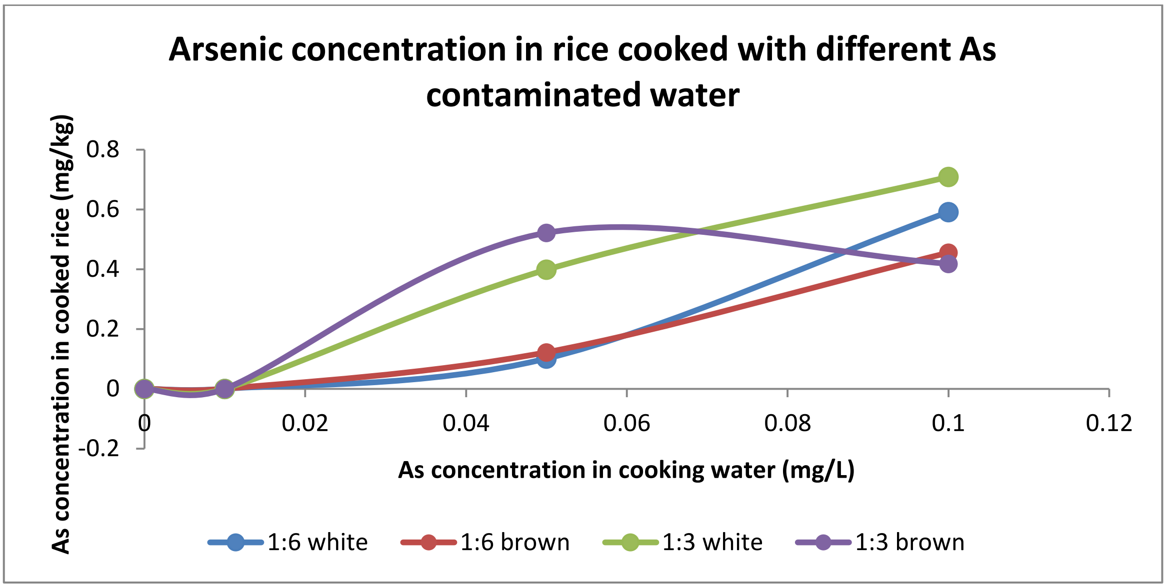

Figure 3.3. The graph above shows the concentration of arsenic retained in cooked rice after cooking in arsenic contaminated water. Arsenic concentration in cooked rice at 0.01 and 0.10 mg/L As water is the average of two replicates.

Percentage decrease in As retained in rice was observed at 0.01 mg/L (**Table 3.6**). At 0.05 and 0.10 mg/L As concentrations, most As was retained in rice after cooking using the 1:3 method compared to the 1:6. However, at 0.10 mg/L brown rice retained more As (500.8%) in the 1:6 cooking method compared to the 1:3 method (452.3%).

Table 3.6. Arsenic retained in rice after cooking in water containing 0.01, 0.05 and 0.10 mg/L As concentration

Rice Variety	**% increase in As concentration from raw rice to cooked rice**					
	0.01 mg/L		**0.05 mg/L**		**0.10 mg/L**	
	1:3	**1:6**	**1:3**	**1:6**	**1:3**	**1:6**
White	-91.8	-91.7	571.9	76.7	1088.7	893.7
Brown	-93.6	-93.6	587.3	65.7	452.3	500.8

3.4.4. Effect of cooking method on micronutrients in long-grain white and brown rice

3.4.4.1. Long-grain white rice

The decrease in the concentration of Mg, Ca, K, P and Fe was observed upon cooking using both methods. A decrease in Mg, Ca, K and P was prevalent in the 1:6 cooking method compared to the 1:3 method. One-way ANOVA was performed on raw and cooked rice and the result showed no significant difference in micronutrients, p = 0.4093.

Table 3.7. Micronutrient composition of long-grain white rice when cooked in arsenic free water (0 mg/L) using 1:3 and 1:6 cooking methods. (n = 1)

Element	**Raw (mg/kg)**	**Cooked**			
		1:3		**1:6**	
		Concentration (mg/kg)	**% decrease from raw**	**Concentration (mg/kg)**	**% decrease from raw**
Mg	259.63	120.11	53.7	85.29	67.1
Ca	35.30	15.51	56.1	10.54	70.1
K	1639.29	849.67	48.2	172.23	89.5
P	1806.22	1200.75	33.5	716.26	60.3
Fe*	13.35	0.49	96.3	9.07	32.1

* Further investigation required

3.4.4.2. Long-grain brown rice

A decrease in K, P and Fe was observed upon cooking using both 1:6 and 1:3 methods. A higher percentage loss in K and P from raw to cooked was seen in rice cooked using 1:6 method compared to 1:3 method. On the other hand, there was increase in Mg and Ca in cooked rice compared to raw rice using both cooking methods. Further investigation will be carried out to establish the cause of this difference in results. One-way ANOVA was carried out on raw and cooked rice and the results showed no significant different in the micronutrients, p = 0.8774.

Table 3.8. Micronutrient composition of long-grain brown rice when cooked in arsenic free water (0 mg/L) using 1:3 and 1:6 cooking methods. (n = 1)

Element	**Raw**	**Cooked**			
		1:3		**1:6**	
		Concentration (mg/kg)	**% decrease from raw**	**Concentration (mg/kg)**	**% decrease from raw**
Mg*	1245.93	1307.66	4.95 (↑)	1257.54	0.9 (↑)
Ca*	116.91	120.77	3.3 (↑)	200.42	71.4 (↑)
K	2250.23	1092.25	51.5	736.77	67.3
P	3685.03	3237.05	12.2	2977.54	19.2
Fe	13.13	10.23	22.1	13.00	0.99

*Further investigation required. (↑): increase

3.5. Discussion

This study was able to investigate total As concentrations in white and brown long-grain rice, otherwise referred to as polished and whole grain rice. The results show that raw brown rice contained high total As when compared to raw white rice. These results agree with those obtained from Meharg *et al*. (2008) study on white and brown rice samples from Bangladesh, China and the U.S. They also suggest that polishing reduces the total and inorganic As content of rice. Hojsak *et al*. (2015) add that rice cultivar, its originality and processing methods also affect the As content in rice. Rice preparation is important in altering the As content of rice. The effect of cooking on As content in rice was studied by Raab *et al*. (2009) and the results revealed that cooking rice in excess water reduced total As concentration by 35-45%. In the current study the 1:6 method caused the most reduction in As concentration in white rice compared to the 1:3 method, 91.8-93.5%. The difference in the percentage of As removed could be due to the fact that 6 different rice varieties were used in Raab's study and average of the total As was calculated were as in the current study, only two rice varieties were used. Generally, the quality of water plays an important role in the overall As content of cooked rice. According to Hojsak *et al*. (2015) cooking rice in uncontaminated water helps to reduce its As content. However, cooking in As contaminated water increases the As retention in rice (Sengupta *et al*., 2006 & Rahman and Hasegawa, 2011). This is of particular concern in areas like Bangladesh were As contaminated rice is cooked in water containing an elevated concentration of As, this increases the burden of As in cooked rice further (Carey *et al*., 2015).

In the current study, the effect of As in cooking water on total As retained in cooked rice was investigated (**Fig. 3.3**). It was observed that the total As retained in the rice cooked in 0.01 mg/L As contaminated water was similar to the one cooked in 0 mg/L (no As) water. However, at 0.05 mg/L As water, a rise in As retained in cooked rice was seen, especially in the rice cooked using the 1:3 method, with percentage increase of 571.9% and 587.3% in white and brown rice respectively. The following highlights the possible reasons; as observed in a study carried out by Sengupta *et al*. (2006), 0 mg/L and 0.01 mg/L As water were considered to be equilibration concentrations therefore there was no increase in As content of rice cooked in these conditions. However, using 0.05 mg/L an increase of 35-40% was seen in the cooked rice. The results obtained for essential elements in this study are in line with the results from a study carried out by Ebuehi and Oyewole (2007), which revealed that cooking and soaking of two rice varieties resulted in the depletion of magnesium, calcium and phosphorus. Although cooking in excess water is important in reducing As retention in rice, a major concern is the removal of trace elements essential for growth and health by exposing the staple to excess water. This is because the surface of the rice grain, about 80 µm thick contains the most concentration of the trace elements (Mihucz *et al*., 2010). This conquers with my results in tables 3.6 and 3.7 were the highest loss of micronutrients in both the white and brown rice varieties were observed in the 1:6 cooking method. The most decrease was observed in Potassium (K), with values of 89.5% and 67.3% in long-grain white and brown rice respectively.

To conclude, the current experiment revealed that As concentrations above 0.01 mg/L in cooking water increase the burden of As in cooked rice. In addition, using excess water as a rice cooking method causes the loss of some essential micronutrients.

Chapter 4

Risk and benefit of different cooking methods on essential elements and arsenic in rice

Material presented in this chapter has been published as:

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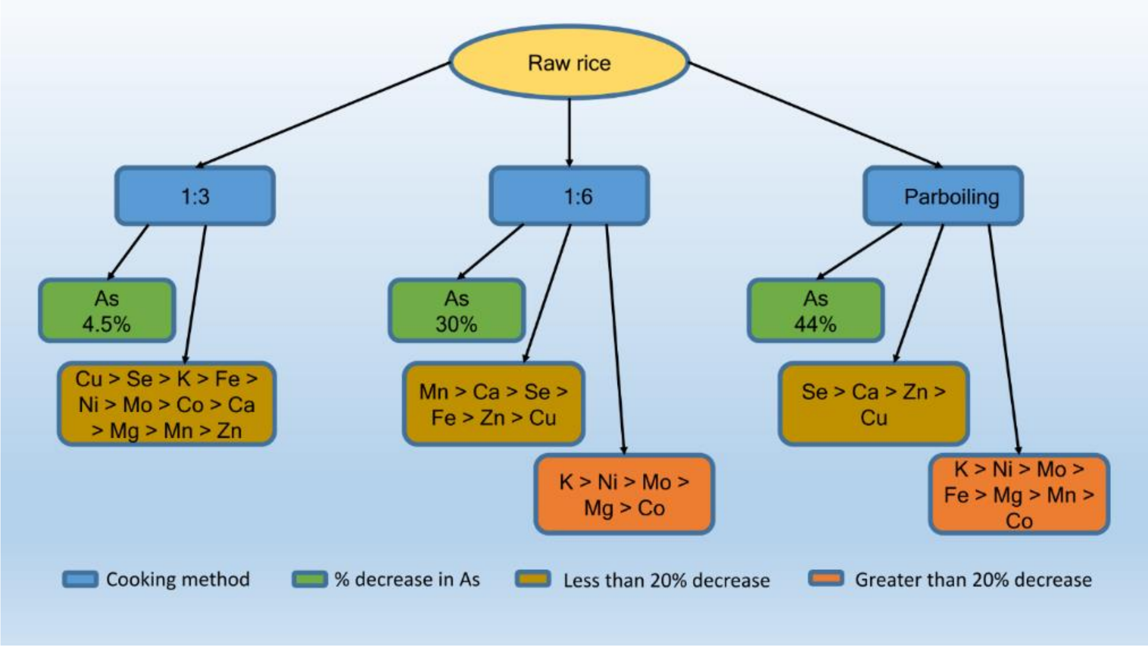


4.1. Abstract

Use of excess water in cooking of rice is a well-studied short-term arsenic removal technique. However, the outcome on the nutritional content of rice is not well addressed. We determined the benefit of different cooking techniques on arsenic removal and the associated risk of losing the essential elements in rice. Overall, we found 4.5%, 30%, and 44% decrease in the arsenic content of rice when cooked with rice-to-water ratios of 1:3, 1:6 (p = 0.004), and 1:10 (parboiling; $p < 0.0001$), respectively. All the essential elements (except iron, selenium, and copper) incurred a significant loss when rice was cooked using the 1:6 technique: potassium (50%), nickel (44.6%), molybdenum (38.5%), magnesium (22.4%), cobalt (21.2%), manganese (16.5%), calcium (14.5%), selenium (12%), iron (8.2%), zinc (7.7%), and copper (0.2%) and further reduction was observed on parboiling, except for iron. For the same cooking method (1:6), percentage contribution to the recommended daily intake (RDI) of essential elements was highest for molybdenum (154.7%), followed by manganese (34.5%), copper (33.4%), selenium (13.1%), nickel (12.4%), zinc (10%), magnesium (8%), iron (6.3%), potassium (1.8%), and calcium (0.5%). Hence, cooked rice is a poor source for essential elements and thus micronutrients.

4.2. Introduction

The genus *Oryza* is composed of about 25 species, cultivated in tropical and sub-tropical regions of Asia, Africa, South America, and Northern Australia and distributed almost entirely across the world (Subudhi *et al*., 2006). In Southeast Asia, both cooked rice grains and processed rice flour are an important part of the daily diet (Akuzawa *et al*., 2002); for example, rice provides around 73% of the calorific intake for the population of Bangladesh (Del Ninno and Dorosh, 2001). In sub-Saharan Africa, rice consumption has increased by more than 50% in the past two decades (Mohanty, 2013). Nigeria in particular has experienced an increase in consumption of about 10% per annum since 1970, and this has been attributed to the change in consumer choice (Akande, 2001). Seifarth (Nzeka, 2018) observed a rise in rice consumption by the Nigerian population due to the ease of accessibility and multiple ways in which it can be prepared. Recently, rice consumption has also increased in Northern and Southern Europe (Maclean *et al*., 2003), and the National Diet and Nutrition Survey (NDNS) carried out between 2008 and 2012 revealed that rice was among the cereals consumed by over 70% of the UK population, thus providing important nutrients and contributing to the diet (Nelson *et al*., 2007; Schenker, 2012).

A variety of factors are important in rice preparation and these govern the quality of the cooked rice. For example, the rice-to-water ratio is a significant aspect and optimal use of water in cooking involves using rice-to-water ratios of between 1:1.5 and 1:2.5 (Chakkaravarthi *et al*., 2008). The traditional method used in Southeast Asia involves a rinsing step and cooking rice in excess water (5–6 times the weight of rice), which is later discarded (Sengupta *et al*., 2006). In the preparation of *Jollof* rice (a popular Nigerian rice dish), excess water is used to boil the rice until a rubbery texture is achieved, similar to parboiling. Thereafter, the rice is rinsed in cold water and added to tomato sauce and ground cray fish, to be cooked to an edible state (Ababio *et al*., 2016).

Despite being widely consumed as a source of carbohydrates, certain vitamins, minerals, and other nutrients including essential elements, rice is an important route of arsenic (As) exposure (Sengupta *et al*., 2006; Pinto *et al*., 2016; Sohn, 2014). Inorganic As is a class 1 carcinogen that has been linked to multiple organ cancers, skin and vascular lesions, and many more health defects (IARC, 2012). According to Hojsak *et al*. (2015), As concentration in rice is higher than in other grains like wheat and barley. The flooded conditions in which it is grown and its ability to absorb As from the soil makes rice the most contaminated cereal compared to other crops (Meharg and Rahman, 2003). However, the concentration of As in rice depends on various factors such as origin, variety, and cooking method. For example, rice is found to be a major source of As exposure in Southeast Asia and can become the most important route in some areas where it is cooked with naturally occurring As-contaminated water (Mondal and Polya, 2008; Mondal *et al*., 2010). However, simple cooking methods can remove arsenic from the grain (Sohn, 2015) and multiple studies suggest that use of excess water for cooking plays an important role as a short-term As removal technique, and a decrease in As of between 15 and 63% has been observed in different studies when rice is cooked with As free water (Sengupta *et al*., 2006; Mihucz *et al*., 2007; Raab *et al*., 2009). However, cooking in excess water also results in the loss of nutrients including essential elements (Gray *et al*., 2015). A loss of 40–75% iron (Fe) depending on the type of rice and cooking technique is reported (Gray *et al*., 2015).

Hence, the nutritional value of rice can depend on the cooking habit adopted by different communities in different countries. This is of particular importance in developing countries where rice is the main staple and micronutrient deficiency, sometimes referred to as ‘hidden hunger’ is prevalent (Tidemann-Andersen *et al*., 2011). The present study determines the effect of three popular rice cooking methods on As and essential elements in rice collected from UK, Sri Lanka, Myanmar, and Nigeria. The contribution of rice cooked by different methods toward the recommended daily intake (RDI) of essential elements is also investigated. To the best of our knowledge, this is the first study comparing how the benefits of cooking rice to remove As can be detrimental due to the loss of essential elements, which can significantly affect the nutritional uptake in the population of developing countries subsistent on a rice-based diet.

4.3. Materials and methods

Rice samples (either whole grain or polished) were collected from four different countries. Among 24 rice samples tested in this study, 11 were from Sri Lanka, 3 from Myanmar, 8 from Nigeria, and 2 (of multiple origin) were purchased from a local superstore in Manchester, UK.

4.3.1. Rice preparation

Ten grams of each rice sample were weighed into a 150 mL beaker and rinsed with 45 mL of deionized (DI) water until the rinse water was clear. Washed rice was subjected to three cooking methods at 385 °C on a hot plate. The first method, known as the contemporary technique (Sengupta *et al*., 2006) involved cooking rice in 30 mL of DI water (the 1:3 ratio) until all the water was absorbed and/or evaporated. The second method, popular in Southeast Asia and referred to as the traditional method (Sengupta *et al*., 2006), required 60 mL of DI water (the 1:6 ratio), and the residual water was discarded once the rice was cooked. During the first two methods, rice was cooked for 10 min or until it was soft to touch. The third method was a type of parboiling, commonly used in Nigeria to cook the popular rice dish known as *Jollof* rice. This type of parboiling involves partially cooking rice in excess water until a rubbery texture is achieved, after which the water is discarded, a tomato stew is added and the rice is left to cook to an edible state. It is different from normal parboiling which requires rice to be soaked, steamed and dried before it is de-husked.

In this method, the washed rice was cooked in 100 mL of DI water (parboiling method, 1:10) for approximately 5 min until it was slightly tender but inedible. The residual water was then discarded.

Cooked rice samples were dried in an oven at 40 °C for 24 h and thereafter in 110 °C oven until constant weight was achieved. The dried rice grains were milled to a semi-powdered form using a mortar and pestle, packaged into resealable bags and stored in a desiccator before being shipped, for analysis to the University of Newcastle, Australia.

4.3.2. Sample Preparation for Elemental Analysis

Rice samples were digested for the analysis of total As and other elements (Fe, calcium (Ca), cobalt (Co), copper (Cu), magnesium (Mg), manganese (Mn), molybdenum (Mo), nickel (Ni), potassium (K), selenium (Se), and zinc (Zn)) based on the protocol of Rahman *et al*. (2009). The determination of As and other trace metals was carried out with an Agilent 7900 (Agilent Technologies, Tokyo, Japan) inductively coupled plasma mass spectrometer (ICP-MS) coupled with an autosampler (Agilent Technologies). Major elements such as Ca, Fe, K, and Mg were analyzed using the dual view (Axial and radial) inductively coupled plasma emission spectrometer (ICP-OES, PerkinElmer Avio 200). CRM, blanks, duplicates, and continuing calibration verification (CCV) were included in each batch throughout the elemental analysis.

4.3.3. Estimated daily intake (EDI) of essential elements and contribution to recommended dietary intake (RDI)

The EDI of each essential element from consumption of rice was estimated using Equation (1)

$$\mathrm{EDI} = \frac{C_{element} \times \mathrm{IR}}{1000} \qquad (1)$$

where $C_{element}$ is the concentration of an essential element (mg/kg) and IR is the ingestion rate (g d^{-1}) of rice, considered to be 100 g per day according to the United States Department of Agriculture (USDA) recommendations.

$$\% \cdot \text{contribution to RDI} = \frac{\mathrm{EDI}}{\mathrm{RDI}} \times 100 \qquad (2)$$

The percentage contribution of each element to RDI was calculated (Equation (2)) using the EDI values. The RDI values were obtained from the USDA Food and Nutrition Board, Institute of Medicine, National Academies website (USDA, 2018). For a particular gender, the highest possible RDI among the different age groups (RDI varies by the age) was used in this calculation. For each essential element measured in rice, we determined the percentage contribution to the RDI for each of the three different cooking methods.

4.3.4. Data analysis

Statistical software STATA (Special edition 11.2, StataCorp LP, College Station, LP, USA) and GraphPad InStat (version 3.1, San Diego, CA, USA) were used for the data analysis. All the results were expressed as mean and standard deviation (Std. Dev). Spearman's rank correlation (r) was used and paired non-parametric Wilcoxon test was performed to determine whether the differences observed in the concentration of As, and the essential elements in raw and cooked rice were significant.

4.4. Results

4.4.1. Quality control analysis

Percentage recovery of As and other elements in the rice flour certified reference material NIST 1568b (n = 6) were as follows: As 110%, calcium (Ca) 107%, cobalt (Co) 101%, copper (Cu) 132%, iron (Fe) 89%, potassium (K) 88%, magnesium (Mg) 80%, manganese (Mn) 97%, molybdenum (Mo) 92%, selenium (Se) 120%, and zinc (Zn) 86%. The limit of detection (LOD) and limit of quantification (LOQ) of each element in the solution matrix are presented in **Table 4.1** below.

Table 4.1. Limit of detection (LOD) and limit of quantification (LOQ) values for As and essential elements.

	As	**Ca**	**Co**	**Cu**	**Fe**	**K**	**Mg**	**Mn**	**Mo**	**Ni**	**Se**	**Zn**
	µg/L	**mg/L**	**µg/L**	**µg/L**	**mg/L**	**mg/L**	**mg/L**	**µg/L**	**µg/L**	**µg/L**	**µg/L**	**µg/L**
LOD	0.01	0.05	0.05	0.02	0.01	0.1	0.05	0.01	0.05	0.1	0.2	0.01
LOQ	0.03	0.17	0.17	0.07	0.03	0.3	0.17	0.03	0.17	0.33	0.67	0.03

4.4.2. Raw rice

Arsenic and other essential elements in raw rice are shown in Table 5.2. Overall, As concentration in raw rice (n = 24) was found to be 0.132 ± 0.10 mg/kg, with an average concentration higher in UK rice samples (0.25 ± 0.02 mg/kg) and lowest in Nigerian rice (0.1 ± 0.097 mg/kg). Furthermore, the relationship between As and essential elements was investigated. The results revealed a significant ($p < 0.05$) positive correlation between As and Mo (r = 0.46), Mg (r = 0.49), K (r = 0.62), and Fe (r = 0.50). There was also a positive correlation between As and Ca (r = 0.38, $p < 0.1$).

4.4.3. Effect of cooking on As in rice

Overall a 4.5%, 30%, and 44% reduction in total As was observed upon cooking rice using the three methods; 1:3, 1:6, and parboiling, respectively (**Fig. 4.1**). Decrease in As was significant for 1:6 (p = 0.004) and parboiling ($p < 0.0001$) techniques. We found the highest reduction in arsenic content in UK rice (52%) followed by rice from Myanmar (42%), Sri Lanka (34%), and Nigeria (9%) when cooked with excess water (the 1:6 rice-to-water ratio). Nigerian raw rice samples had a wide variation in the arsenic content (min 0.01 to max 0.31 mg/kg) and the effect of cooking was not easily detected as most of the samples had very low arsenic concentrations. On parboiling (1:10, rice-to-water ratio), the maximum decrease in arsenic content occurred in UK rice (59%) followed by rice from Myanmar (52%), Sri Lanka (46%), and lastly Nigeria (33%). The differences in the loss rates of As from rice after cooking could be attributed to the different rice varieties (genotypes) apart from the variation due to different sample sizes.

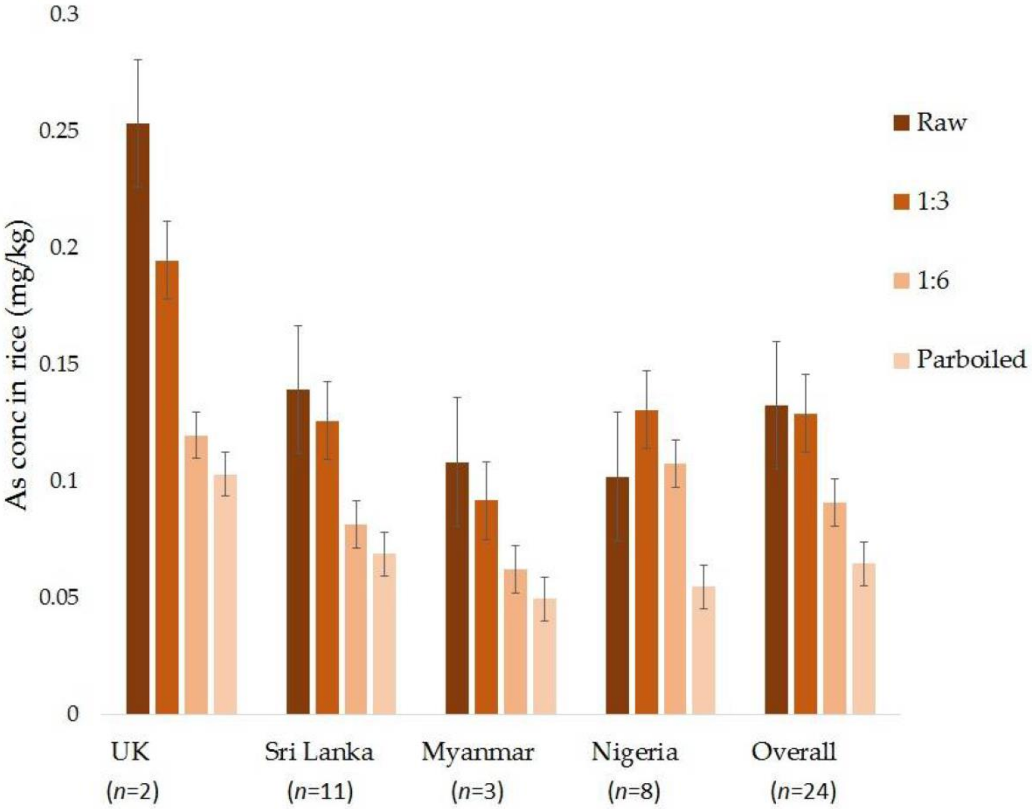


Figure 4.1. Effect of cooking technique on arsenic concentrations in rice samples collected from different countries.

Table 4.2. Total arsenic and concentrations of essential elements (mg/kg) in raw rice.

Location	As	Ca	Co	Cu	Fe	K	Mg	Mn	Mo	Ni	Se	Zn
United Kingdom (*n* = 2)	0.25 ± 0.02	80.64 ± 62.83	0.02 ± 0.01	3.96 ± 0.96	31.00 ± 20.49	1842 ± 342	736 ± 671	19.01 ± 16.05	2.48 ± 2.17	7.23 ± 8.10	0.07 ± 0.03	13.77 ± 5.32
Sri Lanka (*n* = 11)	0.14 ± 0.12	62.13 ± 32.41	0.04 ± 0.02	2.62 ± 0.93	4.67 ± 2.96	1285 ± 439	376 ± 271	8.24 ± 5.27	0.67 ± 0.39	0.22 ± 0.12	0.09 ± 0.04	10.00 ± 3.03
Myanmar (*n* = 3)	0.11 ± 0.03	66.35 ± 9.49	0.02 ± 0.01	3.04 ± 0.66	3.04 ± 0.62	845 ± 124	305 ± 37	7.48 ± 2.00	0.45 ± 0.36	0.20 ± 0.14	0.12 ± 0.14	12.60 ± 0.59
Nigeria (*n* = 8)	0.10 ± 0.10	45.63 ± 10.22	0.04 ± 0.04	3.51 ± 0.63	10.69 ± 12.64	1438 ± 408	247 ± 87	6.03 ± 2.34	0.81 ± 0.22	0.80 ± 0.43	0.06 ± 0.02	8.21 ± 2.71
Overall (*n* = 24)	0.13 ± 0.10	58.70 ± 27.97	0.04 ± 0.02	3.08 ± 0.90	8.67 ± 11.28	1327 ± 447	354 ± 267	8.31 ± 6.10	0.84 ± 0.75	1.00 ± 2.58	0.08 ± 0.06	10.04 ± 3.27
Range	0.01 0.40	22.11 144.36	0.004 0.11	1.39 4.86	1.36 45.49	661 2084	77 1211	3.08 30.36	0.22 4.01	0.06 12.96	0.02 0.28	5.07 17.53

Concentrations are presented as mean ± standard deviation. Sample size is represented by ‘*n*’.

4.4.4. Effect of cooking on essential elements in rice and resultant contribution to RDI

We found a negative correlation between the volume of cooking water and most of the essential elements in the rice samples (**Fig. 4.2**). A significant reduction was observed for all the elements except Cu, Fe, and Se when rice was cooked using the 1:6 ratio and the following trend in percentage reductions was observed: K (50%) > Ni (44.6%) > Mo (38.5%) > Mg (22.4%) > Co (21.2%) > Mn (16.5%) > Ca (14.5%) > Se (12%) > Fe (8.2%) > Zn (7.7%) > Cu (0.2%). Moreover, the method used in the preparation of *Jollof* rice (parboiling) resulted in the further loss of essential elements and the percentage loss to raw rice had the following trend: K (58.9%) > Ni (52.9%) > Mo (52%) > Fe (24.4%) > Mg (23.8%) > Mn (20.8%) > Co (20.4%) > Se (19.3%) > Ca (18.9%) > Zn (14.2%) > Cu (12.5%), with significant decrease for all except Fe. Contemporary cooking (the 1:3 ratio) also resulted in the loss of essential elements but to a much lesser extent compared to 1:6 and parboiling methods.

The contribution to RDI (**Table 4.3**) was highest for rice cooked using the 1:3 ratio followed by 1:6 and parboiling (except for Fe) and the trend for the different essential elements was Mo (154.7%) > Mn (34.5%) > Cu (33.4%) > Se (13.1%) > Ni (12.4%) > Zn (10%) > Mg (8%) > Fe (6.3%) > K (1.8%) > Ca (0.5%) for the 1:6 ratio. This trend was similar for both 1:3 and parboiling methods.

Table 4.3. Percentage contribution of cooked rice to the recommended daily intake (RDI) of essential elements when cooked using the three different methods.

			Cooking Technique		
Essential Element	**Gender**	**RDI (mg/day)**	**1:3 (%)**	**1:6 (%)**	**Parboiled (%)**
Ca	M	1000	0.55	0.49	0.48
	F	1200	0.46	0.41	0.4
Cu	M & F	0.9	33.8	33.4	29.5
Fe	M	8	10.9	8.8	23.8
	F	18	4.8	3.9	10.6
K	M & F	3510	3.6	1.8	1.6
Mg	M	420	8.4	7.0	6.9
	F	320	11.1	9.1	9.1
Mn	M	2.3	33.8	30.3	28.8
	F	1.8	43.2	38.7	36.9
Mo	M & F	0.045	156.8	154.7	147.9
Ni	M & F	1	6.7	12.4	14.2
Se	M & F	0.055	12.4	13.1	11.9
Zn	M	11	8.6	8.4	7.9
	F	8	11.8	11.6	10.8

Co is not included in the RDI calculation because it is not amongst the list of essential elements recommended by the USDA; M: Male; F: Female; mg/day: milligram per day.

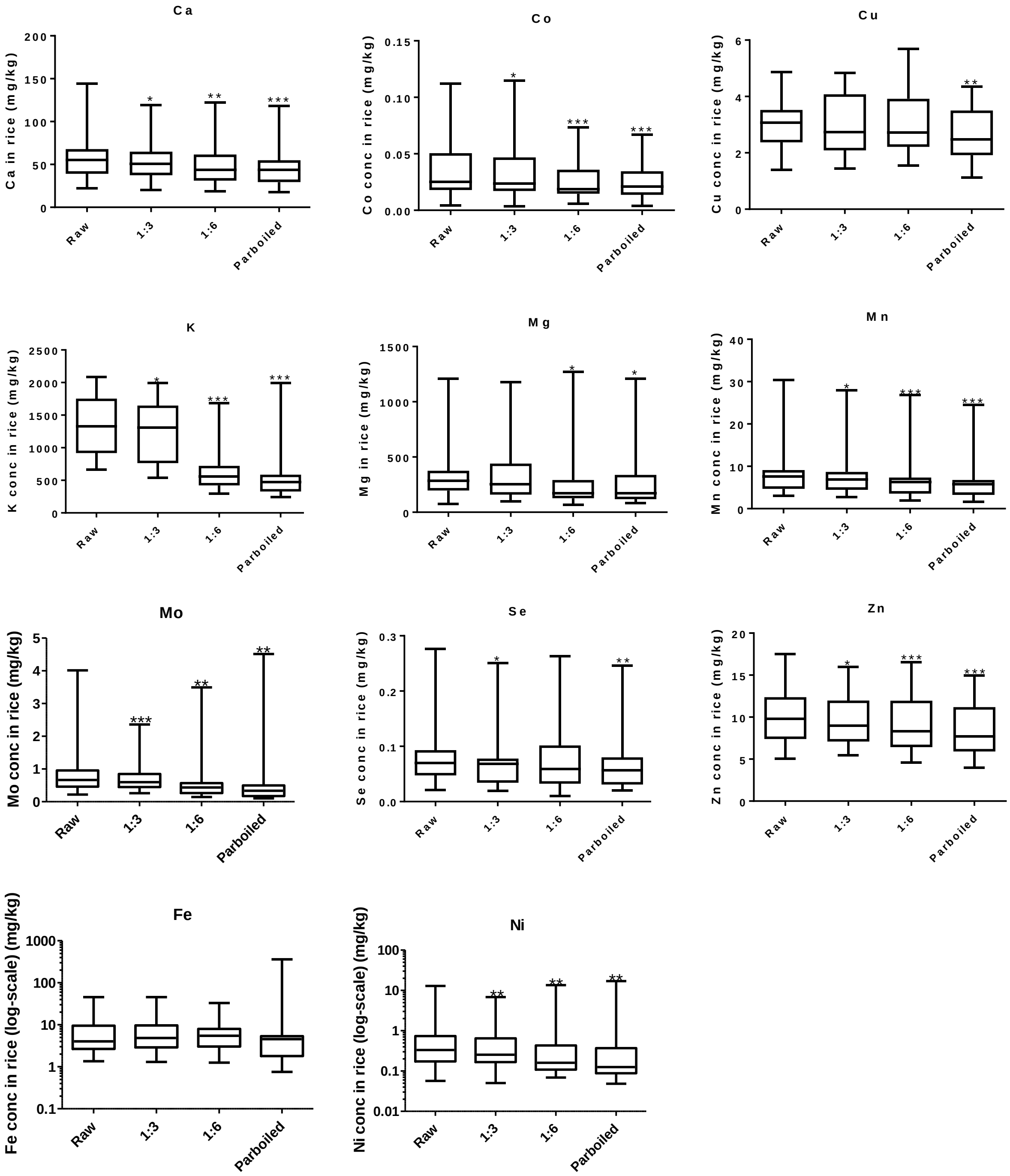


Figure 4.2. Effect of cooking technique on elemental concentrations in rice. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. Paired non-parametric Wilcoxon test was performed to determine the significance in raw and cooked rice. Each box represents the interquartile range (25th and 75th percentile); the band near the middle of the box is the 50th percentile (the median), the whisker represents the 5th and 95th percentile.

4.5. Discussion

The Joint FAO-WHO Codex Alimentarius Commission in July 2014 established a maximum level of 0.2 mg/kg for inorganic As in polished rice (EFSA, 2014) but in a previous study Banerjee *et al*. (2013) reported elevated genotoxic effects in a population from West Bengal, India, consuming cooked rice with total As greater than 0.2 mg/kg. In this study, six out of 24 raw rice samples had total As greater than 0.2 mg/kg. Considering 10–90% of these could be inorganic arsenic (Rahman *et al*., 2014), most of the rice samples had inorganic arsenic below the FAO guideline. When cooked using a rice-to-water ratio of 1:3, the most common method used in Western countries (Sengupta *et al*., 2006), though we observed an overall decrease of 4.5%, one of the Nigerian rice samples (0.27 mg/kg), three from Sri Lanka (0.24, 0.25, and 0.31 mg/kg), and one (0.22 mg/kg) out of the two UK samples had an arsenic concentration of more than 0.2 mg/kg, the threshold observed in the Banerjee *et al*. (2013) study. Moreover, the Nigerian sample, which had 0.31 mg/kg As in raw rice and 0.27 mg/kg in cooked using the 1:3 ratio, had 0.23 mg/kg when cooked using the 1:6 ratio. The rest of the rice samples had an As concentration of less than 0.2 mg/kg when cooked using the 1:6 ratio, with an overall decrease of 30%. Cooking rice in excess water (1:6) is known to reduce As content by 35% (Raab *et al*., 2009), 57% (Sengupta *et al*., 2006), between 15 and 50% (Gray *et al*., 2015), and up to 63% (Mihucz *et al*., 2007). This traditional method is still used by more than 90% of villagers in Southeast Asian regions such as Bangladesh and the Bengal delta of India (Sengupta *et al*., 2006), one of the worst arsenic-affected areas in the world. In a recent study (Gray *et al*., 2015), cooking with an excessive volume of water (a 1:10 rice-to-water ratio) was found to reduce total As content by about 30% for polished long and medium rice grain, 65% for parboiled and 45% for brown rice. Normally, parboiling is a treatment practiced in many Asian and African countries to gelatinize the starch of rice and can be done by different methods (Kwofie and Ngadi, 2017). However, the method used in this study is usually practiced in West Africa, as mentioned earlier. While previous studies have largely reported the effect of different cooking methods on parboiled rice samples (Gray *et al*., 2015; Rahman *et al*., 2006), to the best of our knowledge, this is the first study looking at the effect of parboiling to prepare *Jollof* rice on the As content of rice. Though we observed an overall 44% reduction, the lowest decrease was for Nigerian rice (33%) where this preparation is common.

Amongst all the essential elements that were analyzed in the current study, we observed a positive correlation between As and Mo, Mg, K, Fe, and Ca in raw rice. Previous studies reported similar correlations between As and K, Mg, Mn, and Fe (estimated using Tables 2 and 3 in Pinto *et al*. (2016) and between As and Ni, Se, and Zn (estimated using Table 2 in Somella *et al*. (2013). A significant loss of elements was noted when rice was cooked using the three different methods, the concentrations essentially decreasing as the volume of cooking water increased. Loss of essential elements observed in rice after the use of the 1:3 cooking technique could be attributed to the washing, prior to cooking. At this stage elements were washed away from the surface of the rice grains. According to Mihucz *et al*. (2010), the loss of essential elements was enhanced by their location on the surface of the rice grain, which makes them susceptible to easy removal through washing and cooking. Among all essential elements, the maximum loss was observed for K due to cooking. The concentration of K ranged from 661 to 2084 mg/kg in raw rice, with the highest concentration found in UK rice (1842 mg/kg, **Table 4.2**) followed by the Nigerian rice (1438 mg/kg). However, Nigerian rice samples suffered the maximum loss both after cooking with excess water (1:6; 58.3%) and parboiling (used for *Jollof* rice; 67.8%). In a recent study on mineral composition of commonly consumed local foods in Nigeria, authors reported a low K in *Jollof* rice and mentioned that K was below the recommended levels in the analyzed food samples (Morakinyo *et al*., 2016). The essential element that was least affected by cooking was Cu. The concentration of Cu in raw rice ranged from 1.39 to 4.86 mg/kg, with the highest concentration in UK rice (3.96 mg/kg, Table 4.2) and the lowest (2.62 mg/kg) in Sri Lankan rice.

A decrease in the contribution of essential elements to the RDI was observed with an increase in rice cooking water, except for Fe (**Table 4.3**). Overall, results revealed that Mo contributed the most and in fact more than the required amount to the RDI, 156.8%, 154.7%, and 147.9% when rice was cooked using the 1:3 1:6 ratios and the parboiling method, in spite of the fact that there was substantial decrease in concentration (9.4% for the 1:3 ratio, 38.5% for the 1:6 ratio, and 52% for the parboiling method) due to cooking. UK rice had the highest concentration (2.48 ± 2.17 mg/kg) of Mo in raw rice, whilst Myanmar rice had the lowest (0.45 ± 0.36 mg/kg). A study carried out by Lv *et al*. (2011) on the effect of the environment (air quality, water, and rice) on a population in Zhongxiang, China, revealed that Mo in rice was one of the elements responsible for increasing human health and longevity in the surveyed population. Similarly, Ca and Se in rice were also positively correlated with longevity (Lv *et al*., 2011). However, based on this study, Ca, which contributed the least to the RDI (0.55%, 0.49%, and 0.48% for males and 0.46%, 0.41%, and 0.40% for females for rice cooked with 1:3, 1:6, and parboiling methods, respectively) experienced 8.3% (the 1:3 ratio), 14.5% (the 1:6 ratio), and 19% (parboiling) reductions due to cooking, whilst Se, which was also reduced to a similar extent due to cooking (13.7%, 12%, and 19% via 1:3, 1:6, and parboiling methods, respectively) contributed around 12.4% (the 1:3 ratio), 13% (the 1:6 ratio), and 12% (parboiling) to RDI.

Micronutrients are important for the correct functioning of the body, and a lack of or the presence of imbalancesare associated with disease aetiology (Shenkin, 2006). In addition, insufficient mineral intake can have an effect on our everyday activities, our behaviour, and our physical, intellectual, and emotional states (Damms-Machado *et al*., 2012). Severe cases of Se and Fe deficiency are common all over the world, and low dietary intakes of Mg, Ca, and Zn exist amongst populations in multiple countries (Pinto *et al*., 2016). Iron deficiency is more prevalent in Southeast Asia and Africa, affecting pregnant women, children, and adolescents. Moreover, conditions occurring from micronutrient deficiencies affect over 2 billion people worldwide (Harrison, 2011). Based on our study, it is clear that cooked rice is a poor source of essential elements and thus micronutrients; however, consumed globally, it is the staple for more than half of the world's population (Muraki *et al*., 2015) and is hence a significant source of minerals, especially in certain countries such as rural India and Bangladesh, which are dependent on a rice-based diet (Maclean *et al*., 2003; Pinto *et al*., 2016). According to Maclean *et al*. (2003), micronutrient deficiencies are more severe in areas where rice is a major staple. In poor Asian communities, vegetables are the most popular accompaniments to rice because the population cannot afford, or do not have access to, other types of food, such as meat and fish, from which they obtain additional nutrients (Ricepedia, 2018). Considering a rice consumption rate of 500g/day, (Mondal and Polya, 2008) we found that rice cooked using the 1:6 ratio, which is the traditional method used in Southeast Asia, contributed to 2.5% of Ca, 19% and 9% of Fe for males and females, respectively, 71% and 105% of Zn for males and females, respectively, and 100% of Se based on the RDI of essential elements for Southeast Asia (ILSI, 2018).

The mineral content of rice (depending on the rice variety) is known to be highly influenced by the degree of rice processing such as polishing, milling (Hansen *et al*., 2012), and parboiling (Pinto *et al*., 2016; Kwofie and Ngadi, 2017), but the effect of cooking is less explored. Choice of cooked rice texture differs from one region to another. For example, Das *et al*. (2006) highlighted the different preferences in some parts of the world, stating that countries in the west enjoy long-grain, light, fluffy or slightly dry single rice grains with flavour and no hard core, while Japanese consumers prefer short-grain sticky rice and Indians like medium-grain, light, fluffy individual grains with flavour and a soft core. Hence, methods of rice preparation differ widely.

Our results show that cooking rice in excess water (1:6 and parboiling) reduces the risk of As exposure but results in a reduction of essential elements, thus increasing the risk of micronutrient deficiency, which has severe ramifications especially in children, pregnant women, and the elderly in developing countries dependent on a rice-based diet. We also found that arsenic removal and loss of essential elements due to cooking vary widely depending on the type of rice and its origin.

Chapter 5

Arsenic in rice: a case study

Preliminary study on arsenic in Sri Lankan rice from CKDu endemic areas

5.1. Abstract

Arsenic (As) is believed to play a role in the etiology of Chronic Kidney Disease of unknown origin (CKDu), which has escalated into an epidemic in Sri Lanka. Since, arsenic exposure in Sri Lanka is largely from food intake, we aim to develop a comprehensive overview of As in Sri Lankan rice by comparing the As concentrations in rice collected from three CKDu endemic areas with existing published results. Rice samples were collected from Anuradhapura (n = 4), Trincomalee (n = 3) and Vavuniya (n = 4) and analysed for arsenic along with other trace and major elements. We analysed the correlation between As with cadmium (Cd), lead (Pb), and selenium (Se) by combining all available data published results. Arsenic concentration in rice samples collected from CKDu endemic areas had a wide range of 0.03 to 0.40 mg/kg and to date the highest concentration was found in the samples collected in this study from Vavuniya (0.4 mg/kg). No relationship was observed between As with cadmium and lead and As was not correlated with selenium. Although it is apparent that rice from CKDu endemic areas has As, further investigation is required to establish any relationship.

5.2. Introduction

Rice (*Oryza sativa*) in its grain and processed form is an important staple for over half of the world's population. It is produced and consumed in both developed and developing countries. According to Jayasekera and Freitas (2005), rice and rice based products comprise more than 50% of average daily diet in Sri Lanka. In 2010, the annual per capita rice consumption in Sri Lanka was 116 kg (Jayasumana *et al*., 2015). Rice consumption could be a major source of exposure to arsenic (As), a class I carcinogen, especially in areas where exposure to As from drinking water is low (Mondal and Polya, 2008; Mondal *et al*., 2010) and previous studies have reported As in Sri Lankan rice (Jayasekera and Freitas, 2005; Jayasumana *et al*., 2015; Chandrajith *et al*., 2011; Diyabalanage *et al*., 2016; Mwale *et al*., 2018). Unlike, rice cultivated in As enriched irrigation water in exposed areas of India, Bangladesh, Pakistan, Myanmar, Cambodia, China etc., As in Sri Lankan rice mainly originates from extensive use of fertilizers and pesticides (Jayasekera and Freitas, 2005; Jayasumana *et al*., 2015; Diyabalanage *et al*., 2016).

Recently, there is a strong attention on the quality of food and drinking water especially in areas with prevalent Chronic Kidney Disease of unknown aetiology (CKDu) (Diyabalanage *et al*., 2016). Chronic kidney disease is a non-communicable disease of global public health importance affecting 5-7% of the world's population (Couser *et al*., 2011). CKDu is normally associated with diabetes, hypertension and obesity (Levine *et al*., 2016), however, the exposure to single or multiple nephrotoxicants in the environment has been implicated in the inception and advancement of CKDu, commonly found in North Central Sri Lanka (Redmon *et al*., 2014). This belief has been further enhanced by the geographical distribution of the disease, with higher prevalence in certain areas with specific characteristics (Jayasekara *et al*., 2013) and the kidney disease not being related to any of the known causes (Senevirathna *et al*., 2012). CKDu is a gradually progressive disease and asymptomatic until later stages and is commonly known to affect people of lower socioeconomic background (Levine *et al*., 2016).

In Sri Lanka, CKDu presents a major health and economic burden in rural and agricultural communities (Jayatilake *et al*., 2013) and is reported to kill in excess of 5,000 people each year, with the incidence doubling every four years. Currently, about 2.9 million people are now known to be at risk (Wimalawansa, 2016).

Exposure to heavy metals via consumption of contaminated food is an example of environmental stressor hypothesised to contribute to CKDu risk (Jayasumana *et al*., 2013). But strength of evidence for the role of key suspected heavy metal, As remains inconclusive (Jayasumana *et al*., 2015) while extensively investigated cadmium (Cd) as a putative causative agent in endemic areas have shown mixed findings (Rajapakse *et al*., 2016).

In this study we aim to compare the As concentrations in rice collected from three CKDu endemic areas with previously published results to develop a comprehensive overview of As in Sri Lankan rice. We also explored the relationship of As with other heavy metals like Cd and Pb, and with essential elements in the rice samples.

5.3. Materials and Methods

5.3.1. The rice samples

A total of 11 raw Sri Lankan rice samples were collected from markets in Anuradhapura (n = 4), Trincomalee (n = 3) and Vavuniya (n = 4) and brought back to the University of Salford. The grains were cleaned of sand and soil and 10 g of each sample was washed using deionised water to get rid of any impurities. The samples were then placed in an oven at 40^0 C for 24 hours after which they were dried at 110^0 C until constant weight was achieved. These samples were then ground into powdered form using a mortar and pestle and shipped to Australia for analysis.

Figure 5.1. Image of rice samples obtained from Anuradhapura, Trincomalee and Vavuniya. The image shows husked and polished rice, long and short grains and white, brown and red grains.

5.3.2. Sample preparation for elemental analysis

Rice samples were digested for the analysis of As and other trace and major elements: cadmium (Cd), lead (Pb), calcium (Ca), cobalt (Co), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se) and zinc (Zn), according to the protocol of Rahman *et al*. (2009). The determination of arsenic and other trace metals was carried out by an Agilent 7900 (Agilent Technologies, Tokyo, Japan) inductively coupled plasma mass spectrometer (ICP-MS) coupled with auto sampler (Agilent Technologies), while the major elements were analysed by the dual view (Axial and radial) inductively coupled plasma emission spectrometer (ICP-OES, PerkinElmer Avio 200).

5.3.3. Data analysis

Statistical software STATA (Special edition 11.2, StataCorp LP, USA) and Microsoft excel 2013 were used for descriptive data analysis including determination of Spearman's Rank correlation coefficients and QGIS version 2.18 was used to generate the rice arsenic profile map of Sri Lanka.

5.4. Results

5.4.1. Quality control

Percentage recovery of As and other elements in the rice flour certified reference material NIST 1568b (n = 6) were: As 110%, Cd 91.4%, Ca 107%, Co 101%, Cu 132%, Fe 89%, K 88%, Mg 80%, Mn 97%, Mo 92%, Se 120%, and Zn 86%. The limit of detection (LOD) is presented in **Table 5.1**.

Table 5.1. Limit of detection (LOD) values for As and other elements.

	As (μg/L)	**Cd** (μg/L)	**Pb** (μg/L)	**Ca** (mg/L)	**Co** (μg/L)	**Cu** (μg/L)	**Fe** (mg/L)	**K** (mg/L)	**Mg** (mg/L)	**Mn** (μg/L)	**Mo** (μg/L)	**Ni** (μg/L)	**Se** (μg/L)	**Zn** (μg/L)
LOD	0.01	0.03	0.05	0.05	0.05	0.02	0.01	0.1	0.05	0.01	0.05	0.1	0.2	0.01

Table 5.2 shows the concentration of trace and major elements in the rice samples with highest arsenic concentration found in the samples collected from Vavuniya (0.40 mg/kg). Cadmium was higher in the samples from Trincomalee (maximum: 0.349 mg/kg) and lead was highest (1.98 mg/kg) in the Vavuniyan samples.

Table 5.2. Arsenic and other elements in raw Sri Lankan rice samples collected from three different locations.

Location		**As**	**Cd**	**Pb**	**Ca**	**Co**	**Cu**	**Fe**	**K**	**Mg**	**Mn**	**Mo**	**Ni**	**Se**	**Zn**
Anuradhapura (n = 4)	Mean	0.08	0.013	0.09	40.81	0.04	2.19	3.78	1224	236.13	4.89	0.50	0.20	0.09	8.68
		±	±	±	±	±	±	±	±	±	±	±	±	±	±
		0.07	0.003	0.04	17.25	0.02	0.36	3.22	438	201.07	2.12	0.19	0.07	0.05	2.82
	Range	0.03	0.009	0.04	22.11	0.02	1.69	1.92	661	76.77	3.08	0.31	0.15	0.05	5.93
		0.19	0.017	0.13	63.89	0.07	2.54	8.61	1707	529.03	7.51	0.69	0.30	0.15	11.69
Trincomalee (n = 3)	Mean	0.07	0.125	0.45	53.79	0.03	2.21	2.78	889	243.20	8.20	0.53	0.12	0.09	11.04
		±	±	±	±	±	±	±	±	±	±	±	±	±	±
		0.06	0.190	0.60	15.00	0.01	0.73	1.13	153	97.62	1.60	0.11	0.07	0.04	2.29
	Range	0.03	0.012	0.07	38.92	0.03	1.39	1.48	731	180.55	6.93	0.41	0.06	0.04	8.40
		0.14	0.349	1.14	68.92	0.02	2.77	3.57	1037	355.68	10.00	0.62	0.20	0.12	12.40
Vavuniya (n = 4)	Mean	0.25	0.034	0.73	89.71	0.05	3.36	6.96	1641	615.79	11.62	0.95	0.31	0.10	10.55
		±	±	±	±	±	±	±	±	±	±	±	±	±	±
		0.11	0.032	0.86	36.94	0.02	1.11	2.49	324	278.07	7.50	0.54	0.15	0.05	3.92
	Range	0.13	0.006	0.10	65.86	0.02	2.27	4.33	1258	318.60	4.41	0.50	0.17	0.07	6.96
		0.40	0.076	1.98	144.36	0.07	4.68	9.66	1937	990.38	22.16	1.71	0.50	0.17	15.32
Overall (N = 11)	Mean	0.14	0.051	0.42	62.13	0.04	2.62	4.67	1285	376.12	8.24	0.67	0.22	0.09	10.00
		±	±	±	±	±	±	±	±	±	±	±	±	±	±
		0.12	0.10	0.61	32.41	0.02	0.92	2.96	439	270.83	5.27	0.39	0.12	0.04	3.03
	Range	0.03	0.006	0.045	22.11	0.02	1.39	1.48	661	76.77	3.08	0.31	0.06	0.04	5.93
		0.40	0.350	2.0	144.36	0.07	4.68	9.66	1937	990.38	22.16	1.71	0.50	0.17	12.40

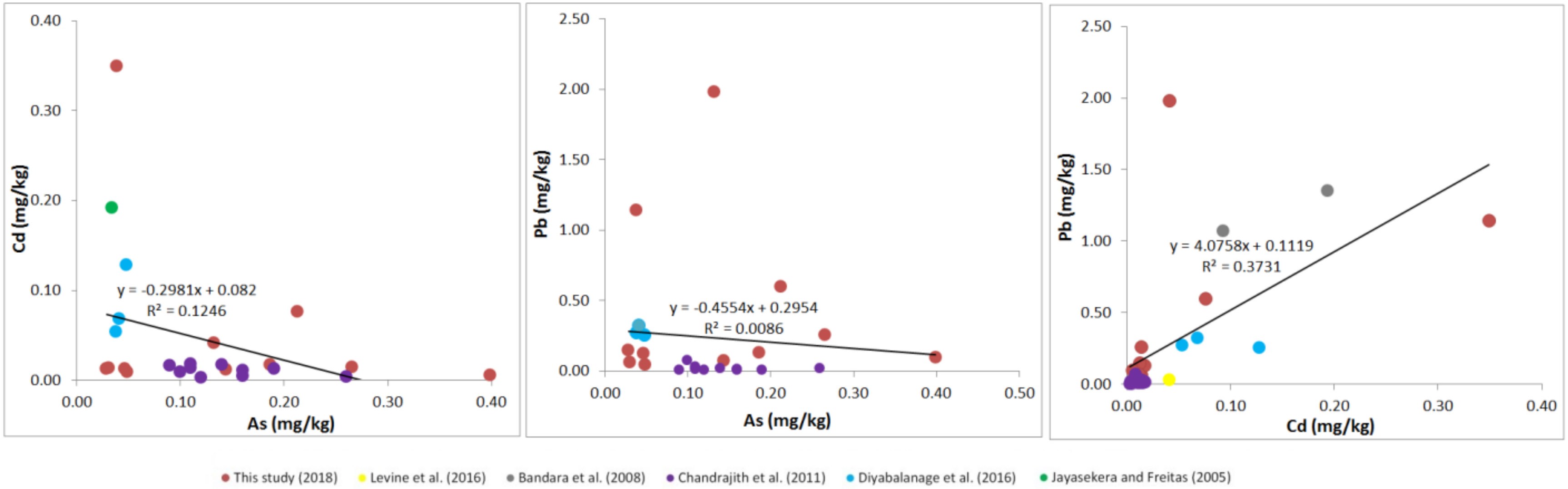


Figure 5.2. Comprehensive overview of correlation between heavy metals in Sri Lankan rice samples. (a) Correlation between As and Cd. (b) Correlation between As and Pb. (c) Correlation between Cd and Pb.

We found significant correlation between cadmium and lead ($r = 0.80$; $p < 0.05$) in the rice samples not only in this study but also including previous publications (**Fig. 5.2**). However, there was no correlation between As and Cd or As and Pb (**Fig. 5.2**). Arsenic was only found to be correlated with K ($r = 0.7$; $p < 0.05$) and Mg ($r = 0.55$; $p < 0.1$) in our rice samples. Selenium was not found to be significantly correlated with any other elements in our rice samples nor was it found to correlate with As, Pb and Cd in the combined data set (**Fig. 5.3**).

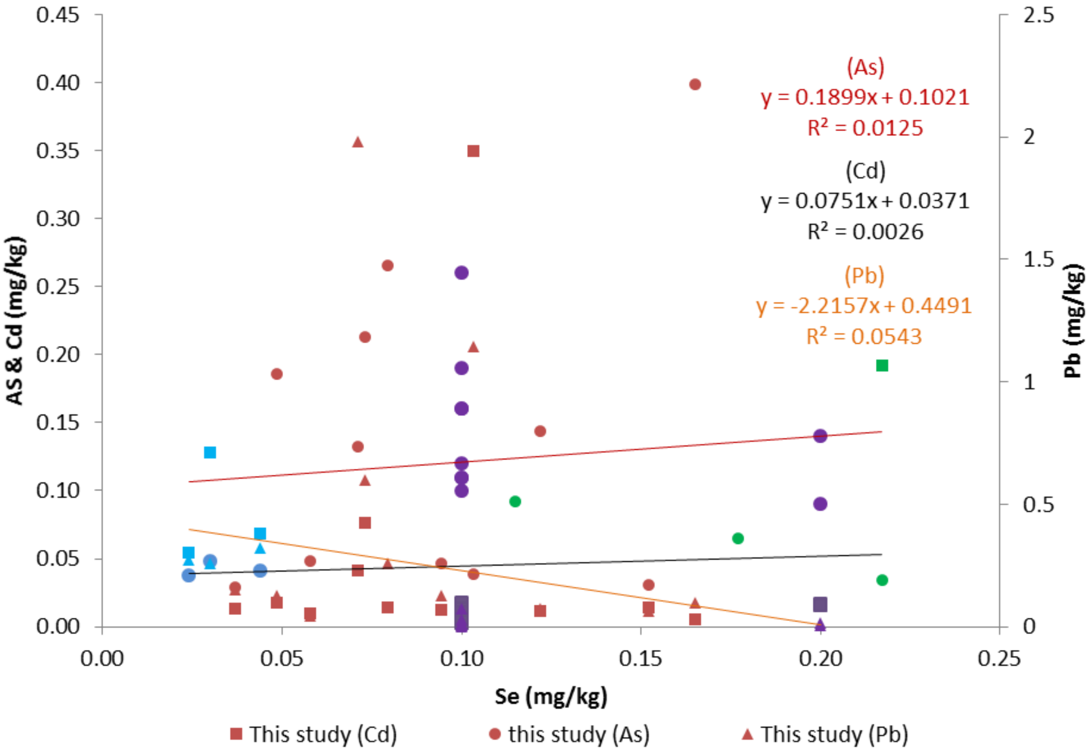


Figure 5.3. Correlation between selenium and heavy metals (As, Pb, Cd) in comprehensive overview of Sri Lankan rice samples. Green represents Jayasekera and Freitas (2005); purple (Chandrajith *et al.*, 2011) and blue (Diyabalanage *et al.*, 2016).

Further correlations between other heavy metals and essential elements are provided in **Table 5.3** below.

Table 5.3. Correlations between heavy metals and essential elements in Sri Lankan rice.

	As	Cd	Pb	Ca	Co	Cu	Fe	K	Mg	Mn	Mo	Ni	Se	Zn
As								0.70*	0.56					
Cd			0.81*											
Pb				0.62*			0.60		0.62*					
Ca							0.75*		0.72*					
Co							0.55	0.70*	0.54					
Cu		0.68*	0.85*											
Fe								0.76*	0.88*					
K									0.80*					
Mg														
Mn			0.71*	0.64*					0.73*					0.85*
Mo		0.55												
Ni		0.65*	0.55			0.75*	0.60				0.59			
Se														
Zn			0.63*											

*p < 0.05, values without symbol: p < 0.10

Although the As concentration of rice samples in this study was similar to that reported by Chandrajith *et al*. (2011) and Jayasumana *et al*. (2015), concentrations reported in Jayasekera and Freitas (2005) and Diyabalanage *et al*. (2016) were lower (**Fig. 5.4**). While Jayasekera and Freitas (2005) was also a market based study, in Diyabalanage *et al*. (2016) arsenic concentrations in rice were reported by zones: wet, intermediate and dry zones. These are representative of the different climatic zones present in Sri Lanka. The wet zone receives over 2500 mm of annual rainfall, intermediate about 1500 mm and finally dry zone about 1000 mm.

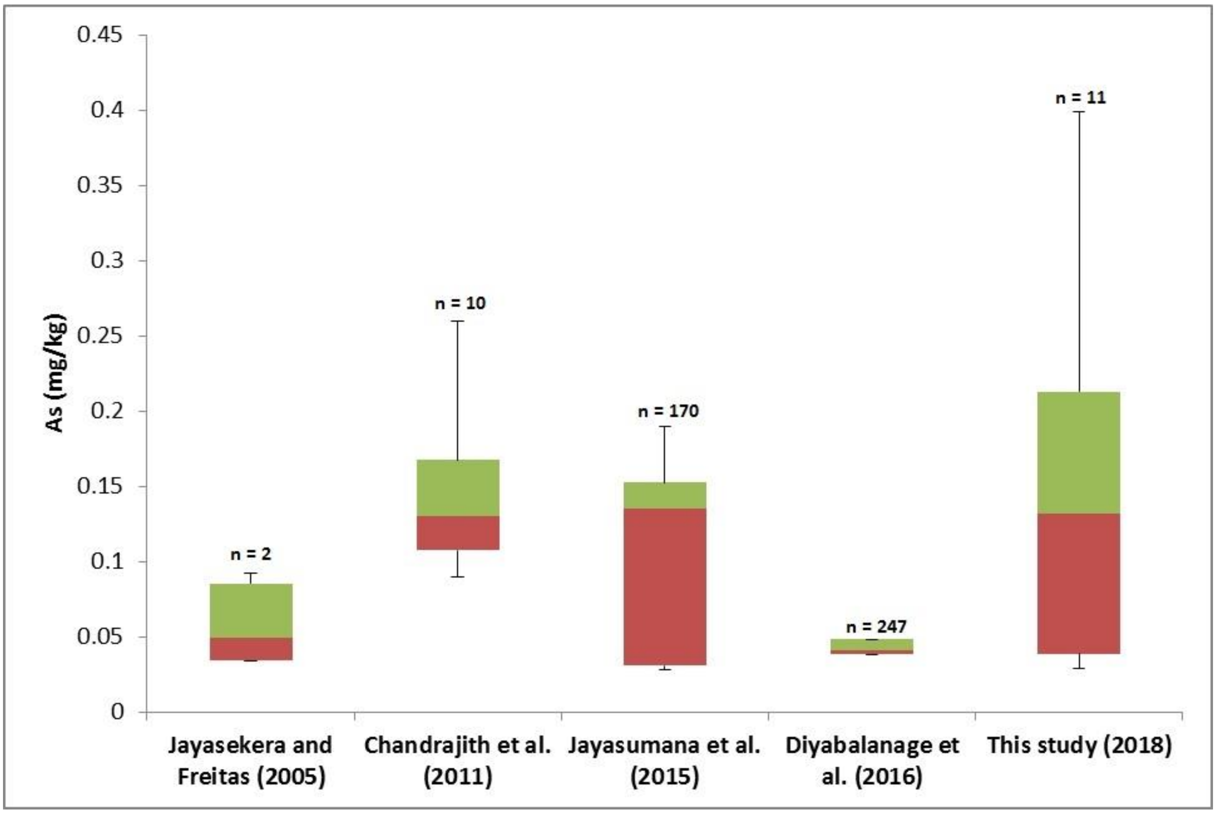


Figure 5.4. Reported arsenic concentration in Sri Lankan rice samples and method of analysis used. Jayasekera and Freitas – raw polished and parboiled gains (Instrumental neutron activation (INAA)), Chandrajith et al. – rice from CKDu endemic areas; Giradurukotte and Nikawewa (Inductively coupled plasma mass spectrometry (ICPMS)), Jayasumana et al. – rice from CKDu areas; Padaviya, Sripura and Mahawilachchiya and non CKDu areas; Kurunegala, Mihinthale, Moneragala and Gampaha (Atomic absorption spectrometry (AAS)) and Diyabalanage et al. – rice samples from wet, intermediate and dry zones (Inductively coupled plasma mass spectrometry (ICPMS)).

Fig. 5.5 presents the rice As concentrations from specific areas as reported in Chandrajith *et al*. (2011) and Jayasumana *et al*. (2015) along with our samples and it shows that all the samples in these studies were collected from CKDu endemic areas except for Gampaha.

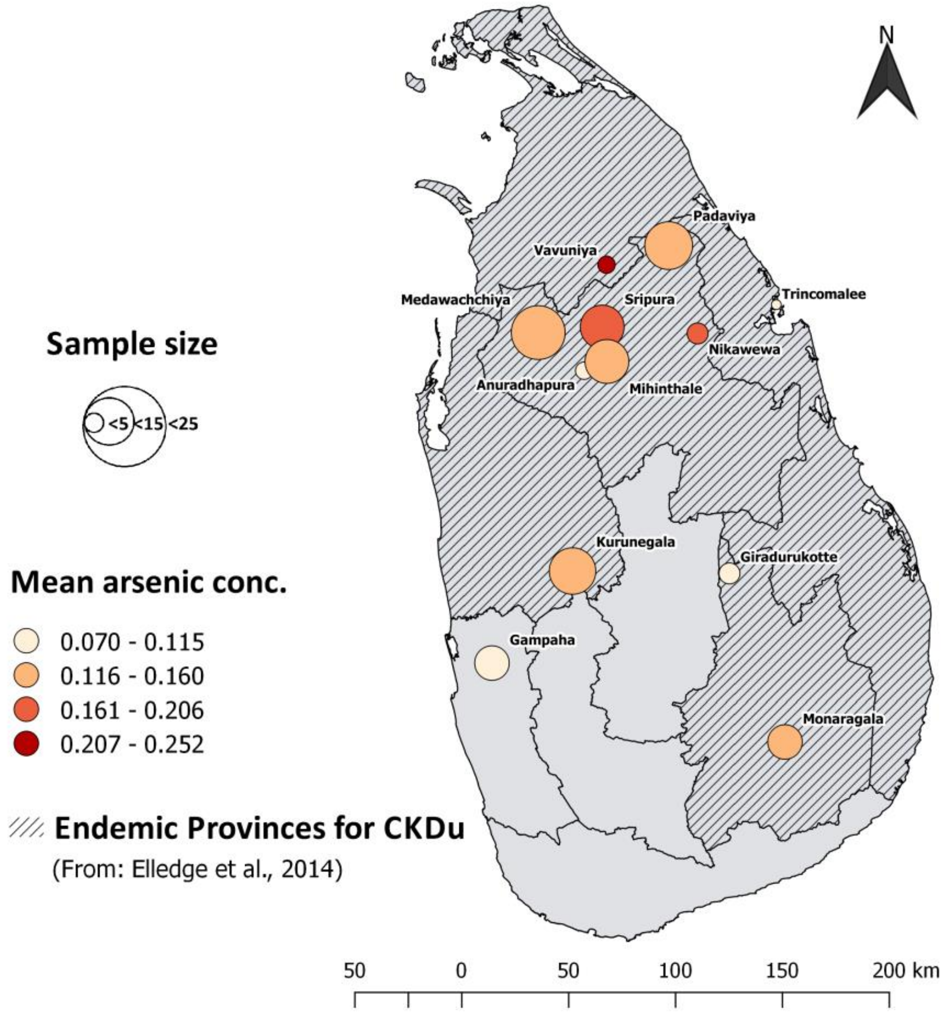


Figure 5.5. Comprehensive overview of As concentration in rice samples collected from CKDu endemic provinces in Sri Lanka. The circles represent sample size. Anuradhapura (n = 4), Trincomalee (n = 3), Vavuniya (n = 4), Giradurukotte (n = 5), Nikawewa (n = 5), Padaviya (n = 20), Sripura (n = 17), Medawachchiya (n = 25), Mihinthale (n = 17), Kurunegala (n = 19), Monaragala (n = 11) and Gampaha (n = 11).

5.5. Discussion

Arsenic is believed to be a causative factor for CKDu in Sri Lanka. Jayasumana *et al*. (2013) observed higher concentration of As in biomarkers of CKDu patients compared to controls. Arsenic exposure in Sri Lanka is predominately from food and previous studies have reported the presence of As in rice (Jayasekera and Freitas, 2005; Jayasumana *et al*., 2015; Chandrajith *et al*., 2011; Diyabalanage *et al*., 2016). When we mapped the As concentrations in rice (**Fig. 5.5**) against CKDu endemic areas (Elledge *et al*., 2014), it is apparent that most of the studies have collected the rice samples from endemic areas but concentration in rice had a wide range from, 0.03 mg/kg in Anuradhapura to 0.40 mg/kg in Vavuniya. While samples collected in this study from Vavuniya had the highest reported As in Sri Lankan rice to date, our sample size was small. In a much bigger, field based study compared to those stated in **Fig. 5.5**, Diyabalanage *et al*., (2016) reported mean As of 0.041 mg/kg (max = 0.186 mg/kg, n = 75) in samples collected from 'dry zone' which approximately covers the whole of CKDu endemic areas. Though the population at risk was found to be scattered in the dry zone, a large number of patients have been detected in Medawachchiya, Padaviya and Girandurukotte with two smaller foci in Medirigiriya and Nikawewa (Jayasekara *et al*., 2013). Mean As concentrations in rice observed in these areas were 0.14 mg/kg, 0.16 mg/kg, 0.11 mg/kg and 0.18 mg/kg for Medawachchiya (n = 25), Padaviya (n = 20), Girandurukotte (n = 5) and Nikawewa (n = 5) respectively (Chandrajith *et al*., 2011; Jayasumana *et al*., 2015).

The wide variation of As in rice collected from CKDu endemic areas could be attributed to study design, for example, market based compared to field based, sample size, and temporal variation. Additionally, the variation in As concentration could be due to different rice varieties and the soil properties for field based studies (Lu *et al*., 2009; Norton *et al*., 2009). Even though based on this study it is apparent that rice from CKDu endemic areas might have As, potential ecological risk of CKDu from As in rice needs further investigation. However, As is a non-threshold carcinogen and severe health effects are well established. While it is difficult to predict the percentage of samples exceeding the stipulated maximum allowable limit for inorganic Asin rice (0.2 mg/kg) as recommended by the joint FAO-WHO Codex Alimentarius Commission (EFSA, 2014), considering 70% of total As in Sri Lankan rice to be inorganic (Jayasumana *et al*., 2015), only one (from Vavuniya) out of the 11 samples in our study exceeded the limit. Based on the reported total As concentrations in three other studies **(Fig. 5.4)** none of the samples had inorganic arsenic (70% of total) above the limit of 0.2 mg/kg.

Since additive effects of As with other heavy metals, especially Cd is also considered as a causative factor for CKDu (Jayasumana *et al*., 2015), we compared both Cd and Pb with As in rice but no correlation was observed (**Fig. 5.2**). All the rice samples had Cd concentrations below the limit of 0.2 mg/kg, set by European Commission (Commission Regulation, 2015) and USDA (for China) (Clever and Jie, 2014) except one from Trincomalee (0.349 mg/kg). Apart from three samples from Vavuniya (0.26, 0.60 and 1.98 mg/kg) and one sample from Trincomalee (1.14 mg/kg) the rest of our samples had Pb concentrations below the maximum limit of 0.2 mg/kg as recommended by the European Commission and USDA (Commission Regulation, 2015; Clever and Jie, 2014). While Pb is found to be strongly correlated with Cd (**Fig. 5.2**) when we combined all existing data for Sri Lankan rice samples, none of the previously reported data had Cd exceeding the limit whereas all the mean Pb concentrations for the three different zones reported by Diyabalanage *et al*. (2016) exceeded the recommended limit and the 'dry zone' had the highest mean concentration of 0.32 mg/kg.
Positive relationship observed between As and K, as well as As and Mg in our study samples is similar to that reported in Somella *et al*. (2013) and Pinto *et al*. (2016), but previous studies on As in Sri Lankan rice did not report essential elements except Jayasekera and Freitas (2005) who determined concentrations of K and Mg in raw polished and parboiled (brown variety) rice samples each collected from two different producers. Although the average As of 0.06 mg/kg (calculated from table 3, Jayasekera and Freitas (2005) was lower than our overall observed mean of 0.14 mg/kg, the calculated (from table 3, Jayasekera and Freitas (2005) average concentrations for K (2046 mg/kg) and Mg (895 mg/kg) were much higher than our overall average of 1285 mg/kg and 376 mg/kg, respectively.

Selenium which is known to exert both synergistic and antagonistic toxicity relationship with As (Sun *et al*., 2014), is not only found to be unrelated with As but also with Pb and Cd in the combined data set (**Fig. 5.3**) incorporating data from Jayasekera and Freitas (2005), Chandrajith *et al*. (2011) and Diyabalanage *et al*. (2016). Overall Se concentration of 0.09 mg/kg in our study samples is lower than previously reported concentration of 0.22 mg/kg in Jayasekera and Freitas (2005) and 0.12mg/kg in Chandrajith *et al*. (2011) but higher than overall average of 0.03 mg/kg calculated from Table 1 in Diyabalanage *et al*. (2016).
In this comprehensive overview of As in Sri Lankan rice we found a wide range of As concentrations in rice samples collected from CKDu endemic areas. No relationship was observed between As with Cd and Pb and As was not correlated with Se.

In this study, we did not consider the nature of As present in the rice samples. Future studies should focus on speciation of As in food. Duplicate diet survey of As and Cd should be carried out to understand the exposure and health risk for the population living in the CKDu endemic areas.

Chapter 6

Arsenic knowledge, practices, attitudes and risk perceptions amongst ethnic and Caucasian groups in the UK.

6.1. Abstract

Rice is a source of nutrients such as carbohydrates, proteins, vitamins, minerals and fibre. However, it is also a major route of As exposure, especially in populations reliant on a rice based diet. Research has shown that cooking method, frequency and amount of rice consumed are all essential in reducing exposure to As from rice intake. This study aimed to identify the risk perception of As exposure from rice intake amongst different ethnic groups and to examine whether knowledge about As contamination has an influence on rice consumption and preparation practices.

A questionnaire survey was carried out to address the As knowledge, rice eating habits, preparation practices and risk perception of 186 participants from the White British, Pakistani, Bangladeshi, African/Caribbean and other white groups. Ethnic minority groups were combined together and referred to as 'grouped ethnicities' for the purpose of data analysis, in which Pearson Chi^2, Fisher's exact test and t-test were employed.

Results from the study revealed that although a higher proportion of the participants had general knowledge of As, very few were aware of As contamination in rice, probably due to the lack of association of As with rice. Prior knowledge of As in rice did not always result in the use of recommended practices involved in rice preparation and consumption. In addition, the White British were more favourably inclined to minimise As exposure from rice by reducing frequency and amount of rice consumption and considering other food options. Thus, suggesting that the other ethnicities perceive low to no risk whilst the White British may perceive risk of exposure to As from rice intake.

6.2. Introduction

Rice is a staple for more than half of the world's population especially in Asia, Africa and some Latin American countries. There has been increase in its consumption in Europe due to its palatability, low-allergenic potential, food diversification and immigration (Akinbile and Haque, 2012; Hite, 2013; Hoogenkamp *et al*. 2017). Current data revealed that 90 grams of rice was consumed per person per week in the UK between 2016 and 2017 and the Bangladeshi communities are by far the largest rice consumer in the UK as compared with other ethnic groups (Statista, 2016; Cascio *et al*., 2010).

Although rice is a source of nutrients such as carbohydrates, proteins, vitamins, minerals and fibre, (Moulick *et al*. 2016; Roy et al. 2011; Torres-Escribano *et al*. 2008; Shraim, 2014), it is also a major route of As exposure (Meharg and Rahman, 2003.; Mondal et al., 2018: Mondal *et al*., 2010). Rice contains higher As compared to other grains like wheat and barley (Su *et al*. 2010; Zhu *et al*. 2008). Arsenic in rice depends on many factors including rice variety (Norton *et al*., 2009), region where it is grown (Lu et al., 2009), irrigation method (Spanu *et al*., 2012) and cooking method (Mandal *et al*., 2018; Mwale *et al*., 2018). Simple cooking method, like use of excess water for cooking can remove As from the grain and plays an important role as a short-term As removal technique (Mwale *et al*., 2018).

Arsenic is a class I carcinogen and can cause skin, bladder, liver, renal and lung cancer in humans (Ahmed *et al*. 2017; International Agency for Research on Cancer, IARC, 2012; Nachman *et al*. 2017). Other health risks include skin lesions, abdominal pain, diarrhoea, diabetes, hypertension, poor mental development, respiratory disorders and cardiovascular diseases (Jitaru *et al*. 2016; Santra *et al*. 2013), hence making it a public health concern.

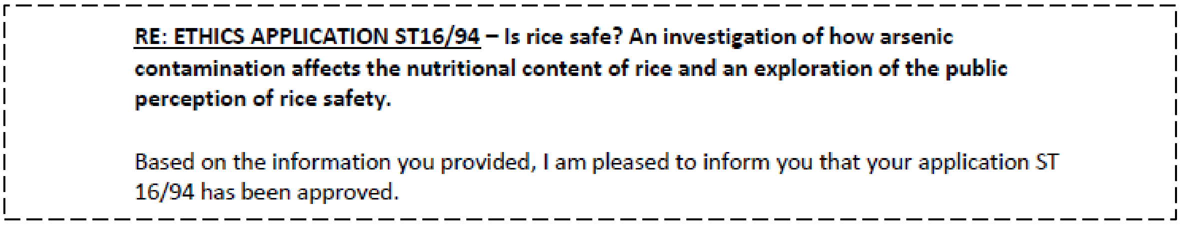
Due to the health risks from As exposure as a result of rice consumption, in 2010, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) appraised that the benchmark dose lower confidence limit for a 0.5% increased incidence of lung cancer for inorganic As (BMDL0.5) should be 3 µg/kg bw per day, which substituted the former limit of 15 µg/kg bw per day, while the European Food Safety Authority (EFSA) set the BMDL0.1 (a 1% increased risk of lung, skin, and bladder cancer) at a range of 0.3-8 µg/kg bw per day (Cubadda *et al*. 2017; Jitaru *et al*. 2016; Rintala *et al*. 2014). In 2014, JECFA recommended a maximum level of 0.2mg/kg of inorganic As in polished rice (white rice) and 0.4mg/kg for brown rice, however, the regulation of As is not enacted in many countries as the recommendation is nonbinding (Jitaru *et al*. 2016; Schmidt, 2015; Signes-Pastor *et al*. 2016; Sergura *et al*. 2016; Stanton *et al*. 2015). Additionally, in the EU the maximum limit of inorganic As in rice-based products is set at 300 µg /kg, while for infants and young children the limit of inorganic As in rice-based products is set at 100 µg /kg (Cubadda *et al*. 2017; Signes-Pastor *et al*. 2017).

The aim of this study was to identify risk perception of As exposure from rice intake amongst different ethnic groups in Manchester, UK and to explore whether knowledge about As contamination has an influence on rice consumption and rice preparation practices.

6.3. Methods

6.3.1. Ethical approval and permission to conduct survey

Prior to the questionnaire survey, ethical approval was obtained from the University of Salford Ethics Committee, as shown below.



RE: ETHICS APPLICATION ST16/94 – Is rice safe? An investigation of how arsenic contamination affects the nutritional content of rice and an exploration of the public perception of rice safety.

Based on the information you provided, I am pleased to inform you that your application ST 16/94 has been approved.

Figure 6.1. This is an extract from the letter sent to the researcher, approving their ethics application.

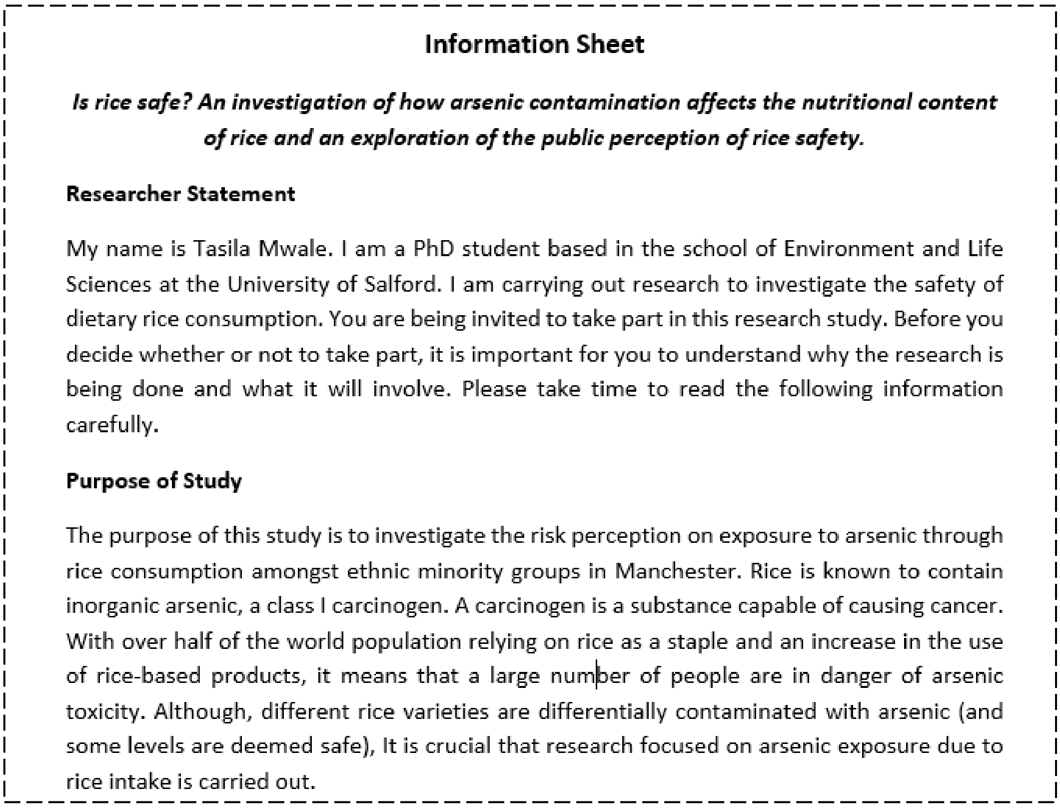
Thereafter, permission to approach participants at the locations of interest was sent to the respective organisations. This was in form of a letter detailing information about the researcher, the study and its purpose, associated risks and benefits and permission to conduct the study at their premises. Contact details were also provided in case they needed further information.

6.3.2. Participants

The questionnaire survey was conducted between December 2016 and April 2017. The study sites were chosen with the help of the 2011 Census data obtained from Manchester city council. Wards containing high percentage of ethnic minorities were selected to target rice eating communities. Moss Side was selected for the Black African/Caribbean population, Longsight for Pakistani and Bangladeshi groups and Moston for the Caucasian. A total of 186 participants were recruited at random from community centres, markets, mother and toddler groups, restaurants and places of worship. Each participant was presented with an information sheet and a consent form before the questionnaire was administered. Only those who gave consent took part in the survey. The study was approved by the University of Salford Ethics Committee (ST16/94).

6.3.3. Obtaining consent

The process of obtaining consent from the participants was divided into two parts. Firstly, the prospective participant was presented with an information sheet (**Fig. 6.2**) detailing the purpose of the study, procedure, associated risks and benefits and confidentiality. In some cases, the researcher read the information to the participants. Secondly, the participant was asked to sign a consent form (**Fig. 6.3**) to signify their agreement to take part in the study. Both of these documents were designed using guidelines provided by the University of Salford.



Information Sheet

Is rice safe? An investigation of how arsenic contamination affects the nutritional content of rice and an exploration of the public perception of rice safety.

Researcher Statement

My name is Tasila Mwale. I am a PhD student based in the school of Environment and Life Sciences at the University of Salford. I am carrying out research to investigate the safety of dietary rice consumption. You are being invited to take part in this research study. Before you decide whether or not to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully.

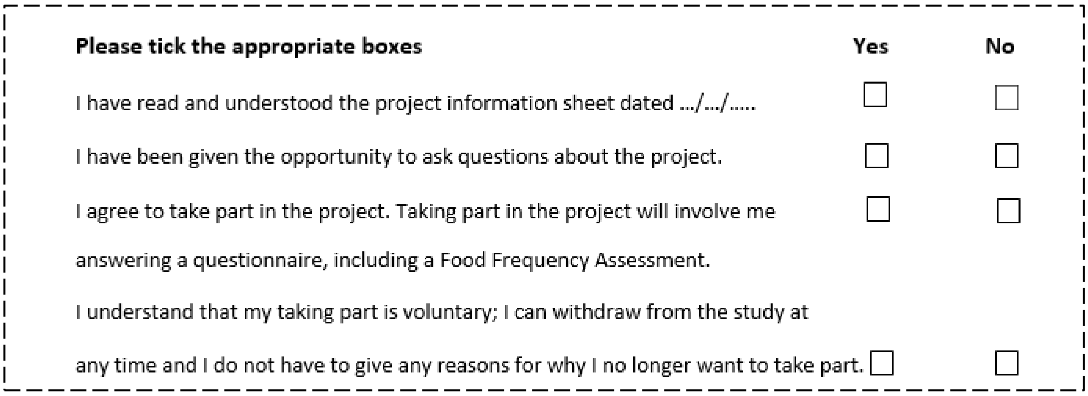
Purpose of Study

The purpose of this study is to investigate the risk perception on exposure to arsenic through rice consumption amongst ethnic minority groups in Manchester. Rice is known to contain inorganic arsenic, a class I carcinogen. A carcinogen is a substance capable of causing cancer. With over half of the world population relying on rice as a staple and an increase in the use of rice-based products, it means that a large number of people are in danger of arsenic toxicity. Although, different rice varieties are differentially contaminated with arsenic (and some levels are deemed safe), It is crucial that research focused on arsenic exposure due to rice intake is carried out.

Figure 6.2. A section of the information sheet inviting participants to get involved in the study.

The information provided to the participants was written in a clear, simple and non-technical way. Time constraints, level of involvement and the rights of the participants were also detailed in the information sheet.

Once the participant had read the information they were asked to complete a consent form. The first part (**Fig. 6.3**) of the consent form included questions which reflected their understanding of the process involved in the study. They were required to answer yes to each question before signing the form.

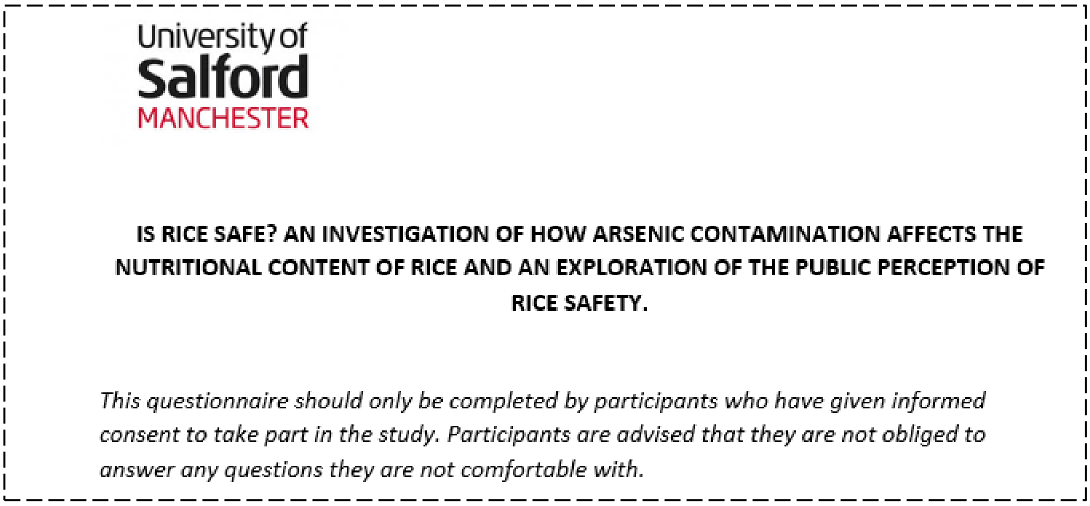


Please tick the appropriate boxes	Yes	No
I have read and understood the project information sheet dated …/…/…..	☐	☐
I have been given the opportunity to ask questions about the project.	☐	☐
I agree to take part in the project. Taking part in the project will involve me answering a questionnaire, including a Food Frequency Assessment.	☐	☐
I understand that my taking part is voluntary; I can withdraw from the study at any time and I do not have to give any reasons for why I no longer want to take part.	☐	☐

Figure 6.3. Questions included on the consent form. The participant must answer yes to each question to indicate that they understand the purpose of the study and are willing to participate.

6.3.4. The questionnaire

The questionnaire (**Fig. 6.4**) included demographic information (age, gender, ethnicity, education, occupation, housing, weekly expenditure on food); questions on a) knowledge of arsenic (Have you ever heard of arsenic?; Do you believe that arsenic is toxic to the human health?) and arsenic in rice (Before this study, were you aware that some rice may contain arsenic?; Do you believe that it is possible for humans to be exposed to arsenic through rice consumption?); b) benefits of rice consumption (Do you think rice is nutritious?; what nutrients can you get from rice?); c) rice consumption pattern (frequency and amount of rice consumed); d) rice preparation practices and attitude (rinsing and ratio of rice to cooking water; Do you believe that cooking rice in excess water can affect nutrients in rice?) and e) risk perception (After today, will you change your rice consumption? Will you change your cooking technique?).



University of
Salford
MANCHESTER

IS RICE SAFE? AN INVESTIGATION OF HOW ARSENIC CONTAMINATION AFFECTS THE NUTRITIONAL CONTENT OF RICE AND AN EXPLORATION OF THE PUBLIC PERCEPTION OF RICE SAFETY.

This questionnaire should only be completed by participants who have given informed consent to take part in the study. Participants are advised that they are not obliged to answer any questions they are not comfortable with.

Figure 6.4. An image of the first page of the questionnaire.

6.3.5. Data collection

A pilot survey was carried out to determine clarity, suitability of terminology and average time required for completion of the questionnaire. Based on the preliminary data from pilot study, modifications were done to ensure comprehensibility of the survey. The questionnaire survey was administered and took approximately 15-20 minutes to complete. Data was collected by three researches, each at different times during the survey period.

6.3.6. Data analysis

Data was analysed using the SPSS version 23 software. Descriptive analysis, frequency and association between variables was carried out for all the data. Pearson Chi^2 cross tabulation, Fisher's exact test and t-test were used to test the significance between the variables. Furthermore, to avoid statistical error due to low population numbers in some ethnic groups, the Pakistan, Bangladeshi, Black African/Caribbean and Other White groups were combined to form one group, referred to as 'grouped ethnicities' whilst the White British was considered as one group/category. The significance levels for the statistical tests were $p < 0.05$ and $p < 0.001$.

6.4. Results

6.4.1. Participant profile

A total of 186 participants took part in the questionnaire survey (**Table 6.1**). 37% were male whilst 63% were female and more female participants were in ‘grouped ethnicities’ (68%) than in Caucasians but it was not statistically significant. Average age of the participants was 44.2 years, with average age of ‘grouped ethnicities’ significantly lower than Caucasians. Though education was significantly different between the two groups (‘grouped ethnicities’ had more participants with higher education), over half (50.3 %) of the participants were educated to at least secondary school level and in terms of occupation both groups were similar. Overall, 55.1% of the participants were tenants and as a mode of housing it was significantly higher in Caucasians. Though most of the participants (57%) spent between £20 and £50 on food per week, there were more participants in ‘grouped ethnicities’ who spend more than £50 compared to Caucasians.

Table 6.1. Participant demographic information.

Demographic Variable	**White British**	**Asian or Asian British: Pakistan**	**Asian or Asian British: Bangladesh**	**Black or Black British: African/Caribbean**	**Other White**	**Grouped ethnicities**	**Total**	**P value**
	(n = 75)	(n = 62)	(n = 31)	(n = 16)	(n = 2)	(n = 111)	(N =186)	
Age: Mean (standard deviation)	48.6 (12.8)	38.6 (12.6)	40.8 (14.1)	53.8 (16.1)	32.5 (0.7)	41.3 (14.3)	44.2 (14.2)	**0.0005 *****
Sex: Count (%)								
Male	34 (45.3)	25 (40.3)	5 (16.1)	4 (25)	1 (50)	35 (31.5)	69 (37.1)	**0.056***
Female	41 (54.7)	37 (59.7)	26 (83.9)	12 (75)	1 (50)	76 (68.5)	117 (62.9)	
Education: Count (%)								
Primary	8 (10.7)	6 (9.8)	1 (4.3)	3 (18.8)		10 (10)	18 (10.2)	**0.000 ****
Secondary	50 (66.7)	25 (41)	8 (34.8)	6 (37.5)		39 (39)	89 (50.3)	
Higher Education (College/University)	17 (22.7)	23 (37.7)	13 (56.5)	7 (43.8)	2 (100)	45 (45)	62 (35)	
Other	0	7 (11.5)	1 (4.3)	0	0	6 (6)	8 (4.5)	
Housing: Count (%)								
House owner	25 (33.3)	29 (47.5)	12 (38.7)	4 (25)	1 (50)	46 (41.8)	71 (38.4)	**0.012****
Tenant	49 (65.3)	28 (45.9)	13 (41.9)	11 (68.8)	1 (50)	53 (48.2)	102 (55.1)	
Other	1 (1.3)	4 (6.6)	6 (19.4)	1 (6.3)	0	11 (10)	12 (6.5)	
Occupation: Count (%)								
Self employed	24 (32)	26 (41.9)	2 (6.5)	0	2 (100)	30 (27)	54 (29)	**0.130****
Employed	23(30.7)	12 (19.4)	7 (22.6)	6 (37.5)		25 (22.5)	48 (25.8)	
Unemployed	17 (22.7)	12 (19.4)	15 (48.4)	3 (18.8)		30 (27)	47 (25.3)	
Student	0	5 (8.1)	2 (6.5)	1 (6.3)		8 (7.2)	8 (4.3)	
Volunteer	2 (2.7)	2 (3.2)	1 (3.2)	3 (18.8)		6 (5.4)	8 (4.3)	
Other	9 (12)	5 (8.1)	4 (12.9)	3 (18.8)		12 (10.8)	21 (11.3)	
Money spent on food weekly: Count (%)								
<£10	0	1 (1.6)	0	0	0	1 (1.1)	1 (0.5)	**0.016****
£10-£20	10 (13.3)	12 (19.4)	5 (16.1)	3 (18.3)	0	16 (17.6)	30 (16.1)	
£20-£50	53 (70.7)	25 (40.3)	17 (54.8)	11 (68.8)	0	43 (47.3)	106 (57)	
£60-£100	11 (14.7)	21 (33.9)	6 (19.4)	1 (6.3)	1 (50)	26 (28.6)	40 (21.5)	
>£100	1 (1.3)	3 (4.8)	3 (9.7)	1 (6.3)	1 (50)	5 (5.5)	9 (4.8)	

Percentage by column and actual numbers in brackets. *= Pearson chi^2 test; **= Fisher's exact; ***= t-test comparing White British with Grouped Ethnicities.

6.4.2. Rice consumption and preparation practices

According to the survey, the variety of rice most consumed by the participants was basmati rice (62.8%). Amongst the basmati consuming population, the Pakistani emerged as the highest (38%) rice consuming community followed by the White British (34%), Bangladeshi (18.7%), African/Caribbean (8.7%) and last but not the least Other White group (0.7%). On the other hand, only 2.1 % of the surveyed population reported consuming the wild rice, which was the least consumed variety. 52% of the participants purchased their rice from the local supermarkets whilst 48 % purchased theirs from African or Asian shops. 67.2% of the participants consumed rice twice a week or less (**Table 6.2**). Overall, the percentage of participants consuming less than 1 cup of rice per serving, 1 cup and 2 cups or more was 12.7%, 39.3% and 48% respectively. The most popular rice accompaniment was meat whilst the least was milk. There was a significant difference in the frequency of consumption between the White British and the combined ethnic groups. A higher percentage of the white British (73.3%) in comparison to the other ethnicities, practiced the recommended frequency of rice consumption (twice a week or less). On the other hand, a higher percentage of the grouped ethnicities (36.9%) consumed rice more than twice a week in comparison to the White British (26.7%). Further investigation revealed that out of all the ethnic groups being studied, a greater proportion of the Bangladeshi (80.6%) consumed rice more than twice a week, in comparison to the other groups. 61.3% of the Bangladeshi population consumed rice at least once a day whilst 19.4% admitted to eating rice two times or more per day. A significantly higher percentage of the White British (36%) consumed greater amount of rice in one serving in comparison to the grouped ethnicities (28.2%).

Rinsing of rice was practiced by majority of the ethnic groups. However, a significant difference was observed between the combined ethnic groups (99.1%) and the White Caucasian (82.1%). The most popular rice cooking method amongst all the participants was the 1:2 or less method; practiced by 80.3% of the White British and 82.1% of the grouped ethnicities. The least popular method involved the use of excess water (> 1:4) by 19.7% of White British and 17.9% of grouped ethnicities. Comparison in cooking technique between White Caucasian and combined ethnic groups was found to be statistically insignificant. A significant majority of the White British and ethnic minority groups revealed that some of their cooking habits were either inherited or influenced by their ethnic background.

Table 6.2. Relationship between ethnicity and rice preparation and consumption.

	White British	Asian British: Pakistani	Asian British: Bangladeshi	Black British: African/Caribbean	Other White	Grouped Ethnicities	Total	P value
Frequency: **count (%)**								0.000*
Twice a week or less (recommended)	55 (73.3)	53 (85.5)	6 (19.4)	10 (62.5)	1 (50)	70 (63.1)	125 (67.2)	
More than twice a week (not recommended)	20 (26.7)	9 (14.5)	25 (80.6)	6 (37.5)	1 (50)	41 (36.9)	61 (32.8)	
Amount: Count (%)								0.010**
<1 cup	10 (13.3)	15 (24.6)	11 (35.5)	5 (31.3)	0	31 (28.2)	41 (22.2)	
1 cup	38 (50.7)	24 (39.3)	16 (51.6)	8 (50)	0	48 (43.6)	86 (46.5)	
2 cups or more	27 (36)	22 (36.1)	4 (12.9)	3 (18.7)	2 (100)	31 (28.2)	58 (31.4)	
Rinsing: Count (%)								0.000**
Yes	55 (82.1)	62 (100)	31 (100)	15 (93.8)	2 (100)	110 (99.1)	165 (92.7)	
No	12 (17.9)	0	0	1 (6.3)	0	1 (0.9)	13 (7.3)	
Rice to water ratio: Count (%)								0.267*
1:2 or less	53 (80.3)	46 (79.3)	23 (76.7))	16 (100)	2 (100)	87 (82.1)	140 (81.4)	
1:4 – 1:6	13 (19.7)	12 (20.7)	6 (20)	0	0	18 (17)	31 (18)	
>1:6	0	0	1 (3.3)	0	0	1 (0.9)	1 (0.6)	
Inherited cooking practices: Count (%)								0.006*
Yes	55 (78.6)	58 (96.7)	28 (90.3)	14 (87.5)	1 (50)	101 (92.7)	156 (87.2)	
No	15 (21.4)	2 (3.3)	3 (9.7)	2 (12.5)	1 (50)	8 (7.3)	23 (12.8)	
Cooking and ethnic background: Count (%)								0.000*
Yes	47 (68.1)	58 (96.7)	28 (90.3)	14 (93.3)	2 (100)	102 (94.4)	149 (84.2)	
No	22 (31.9)	2 (3.3)	3 (9.7)	1 (6.7)	0	6 (5.6)	28 (15.8)	

1:2 (two times more water than rice), 1:4 (four times more water than rice), 1:6 (six times more water than rice). *=Pearson Chi^2 test; **=Fisher's exact.

6.4.3. Awareness of As contamination and rice consumption and preparation practices

When the participants were asked ‘if they had ever heard of As’, 56.5 % (n= 105) answered in the affirmative and all confirmed that they ‘believe As is toxic to human health’. Being male, aged >45 years and employed were significantly associated with As knowledge (**Table 6.3**). 92% of the Caucasians said they knew about As compared to 32% of ‘grouped ethnicities. When this cohort was asked ‘if they believe that it is possible for humans to have As exposure from rice intake’ only 14.5% (n=15) said yes. Among these 15 participants, six were Caucasians and the rest were from ‘grouped ethnicities. The Pakistani ethnic group had the highest number (88.7%) of participants who had never heard of As, followed by the Bangladeshis (58.1%).

There was no significant difference in rice consumption with respect to As knowledge. In terms of cooking practices, while rinsing/washing rice before cooking was the most common practice as stated before, surprisingly, for participants who didn’t rinse their rice, significantly higher percentage were with As knowledge, but numbers are very small to make any judgement. The most popular method of cooking amongst participants with and without As knowledge involved using two times more water than rice (1:2) but there was no significant difference.

Table 6.3. Factors contributing to general As knowledge and relationship with rice consumption and cooking practices.

	Have you ever heard of As?		P value
	Yes	**No**	
Age: Count (%)			
18-24 (n = 16)	4 (25)	12 (75)	0.026***
25-29 (n = 13)	6 (46.2)	7 (53.8)	
30-34 (n = 19)	8 (42.1)	11 (57.9)	
35-39 (n = 27)	16 (59.3)	11 (40.7)	
40-44 (n =24)	9 (37.5)	15 (62.5)	
45+ (n = 87)	62 (71.3)	25 (28.7)	
Gender: Count (%)			
Male	46 (66.7)	23 (33.3)	0.031*
Female	59 (50.4)	58 (49.6)	
Highest Level of Education: Count (%)			
Primary	11 (61.1)	7 (38.9)	0.785**
Secondary	55 (61.8)	34 (38.2)	
Higher Education	38 (61.3)	24 (38.7)	
Other	0 (0.0)	1 (100))	
Occupation: Count (%)			
Self employed	28 (51.9)	26 (48.1)	0.028**
Employed	34 (70.8)	14 (29.2)	
Unemployed	24 (51.1)	23 (48.9)	
Student	1 (12.5)	7 (87.5)	
Volunteer	6 (75.0)	2 (25.0)	
Other	12 (57.1)	9 (42.9)	
Ethnicity: Count (%)			
White British	69 (92)	6(8)	0.000**
Grouped ethnicities	36 (32.4)	75 (67.5)	
Frequency of rice consumption: count (%)			
Twice a week or less (recommended)	70 (56)	55 (44)	0.859*
More than twice a week	35 (57.3)	26 (42.7)	
Amount of rice consumed: count (%)			
<1 cup	21 (51.2)	20 (48.8)	0.349**
1 cup	53 (61.6)	33 (38.4)	
2 cups	28 (57.1)	21 (42.9)	
>2 cups	3 (33.3)	6 (66.7)	
Washing rice: count (%)			
Yes	87 (52.7)	78 (47.3)	0.023**
No	11 (84.7)	2 (15.4)	
Rice to water ratio: count (%)			
1:1	10 (50)	10 (50)	
1:2	69 (57.5)	51 (42.5)	0.822*
1:4 – 1:6	18 (56.2)	14 (43.8)	

Percentage in brackets by row. *= Pearson Chi^2 test; **= Fisher's exact; ***=t-test.

6.4.4. Attitudes and risk perception of As exposure from rice intake

In addition to their current practices, the questionnaire also investigated the attitudes and risk perception of the participants after the study. When asked whether they would change their cooking habits (**Fig. 6.5a**), majority of participants from the White British group (56.1 %) in comparison to the grouped ethnicities (41.6%) said they would. Examples of changes included rinsing and soaking of rice before cooking, the use of excess water during cooking, draining out water when rice is soft.

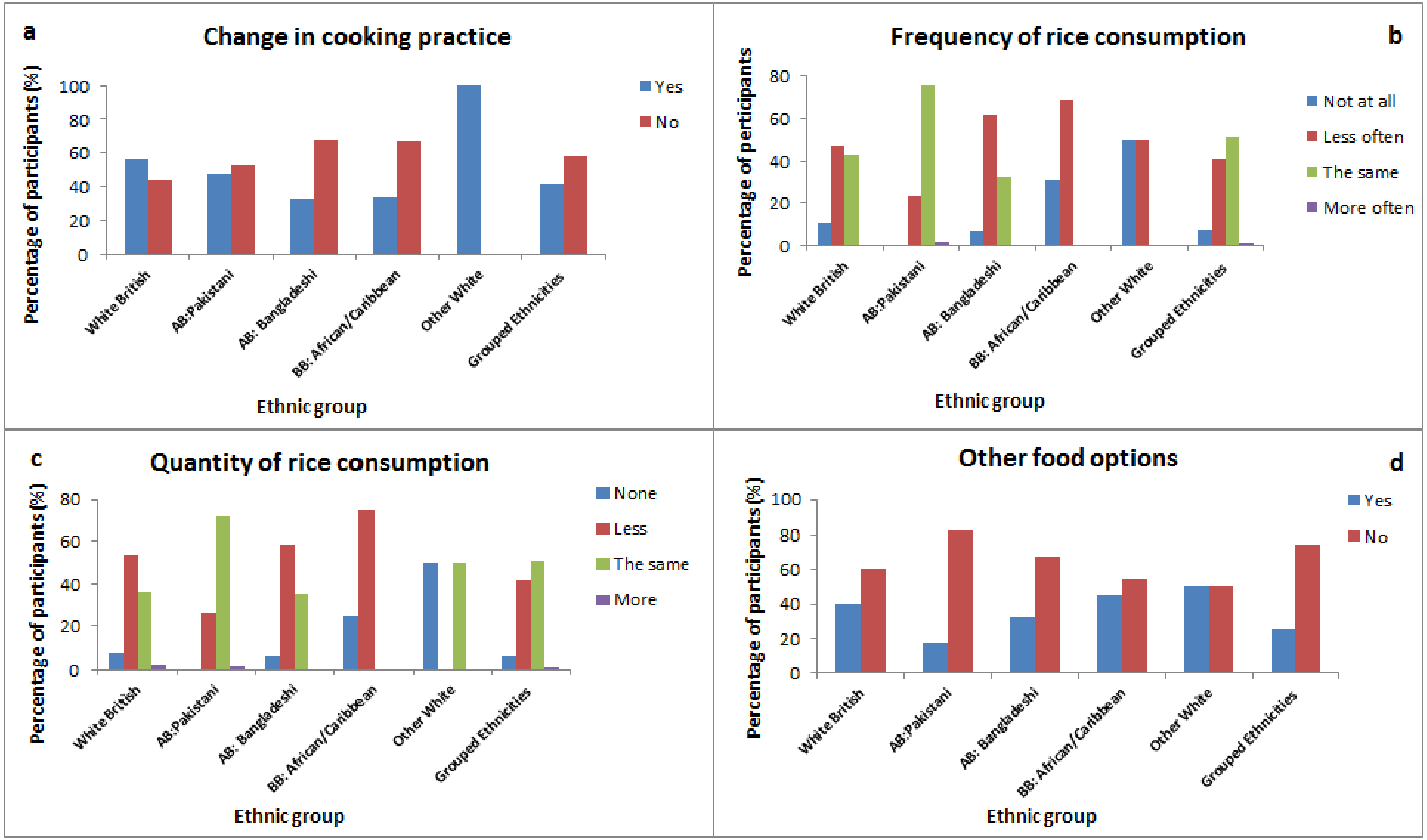


Figure 6.5. Attitudes of ethnic minority groups towards rice preparation and consumption.

Fig. 6.5b represents results from the survey on the attitude of the ethnic groups with regards to frequency of rice consumption after being informed about As contamination in rice. Majority of the White British (46.7%) said they would reduce their frequency of rice consumption whilst a greater proportion of the combined ethnic group (50.9%) revealed that they would not change their frequency of rice consumption. A similar pattern was observed in the answers given to quantity of rice consumed after the study (**Fig. 6.5c**). 53.3% of the White British said they would consume less rice whilst 50.9% of the combined ethnic group said they would not change their quantity of rice consumption. Statistical tests showed no significant difference in the quantity or frequency of rice consumption amongst the different groups ($p > 0.05$). When questioned about considering food options other than rice (**Fig. 6.5d**), the White British (39.7%) were more likely to replace rice with a different food option whilst the grouped ethnicities (25.5%) were less likely to do so.

6.4.5. Other Practices

6.4.5.1. Rice product and grain consumption

Apart from rice consumption, the participants were also questioned about their rice product consumption habits (**Fig. 6.6**). The most popular rice product amongst the participants was rice krispies, consumed by 39.2 % of the population at least once a day. The least consumed rice products were rice drink, rice bran oil and rice wine/beer, consumed by only 1.3 % of the population. It was observed that rice krispies were a popular choice by both participants with and without As knowledge, 60 % and 66.7 % respectively.

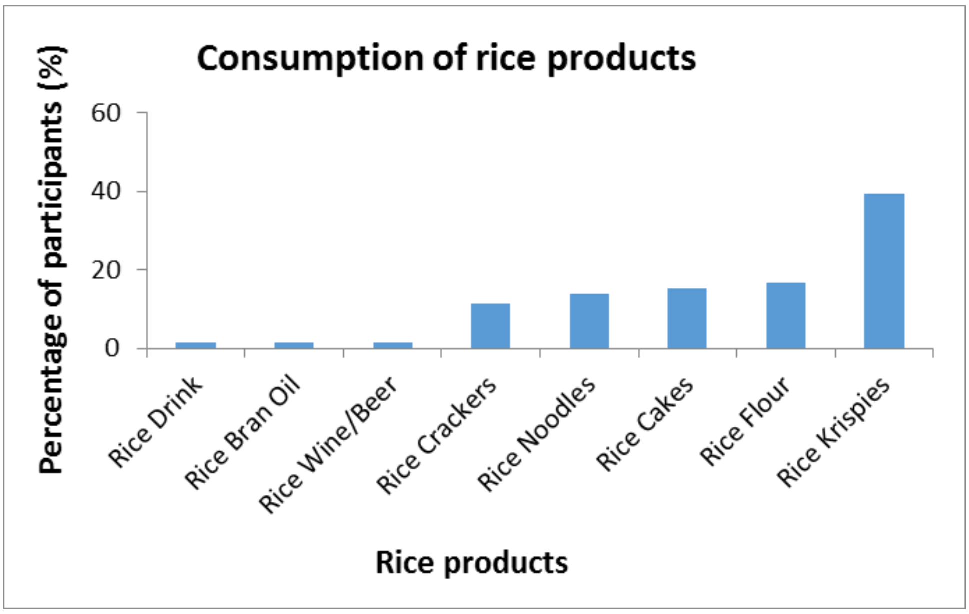


Figure 6.6. Consumption of rice products amongst the participants.

Fig. 6.7 below is a representation of grain consumption amongst the survey participants. Apart from rice, another grain consumed the most by the participants is corn (30.7 %) followed by oats (26.6 %) and the least rye, consumed by only 0.3 % of the population.

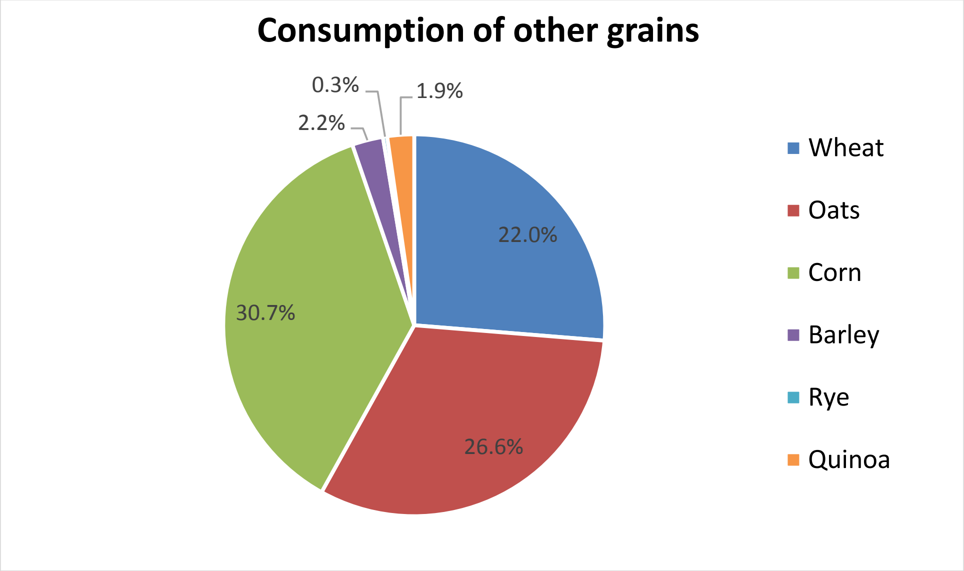


Figure 6.7. Grain consumption amongst the participants.

6.4.5.2. Breakfast, lunch and dinner choices

In order to gain more understanding about the pattern of rice and rice product consumption at different times of the day (**Fig. 6.8**), the participants were questioned about their food choices at breakfast, lunch and dinner. For breakfast, the most popular food option was bread (43 %) and the least popular was rice (3 %). 35.1 % of the participants said they consume cereals for breakfast. However, it is impossible to determine if any of the cereals were produced from rice because the survey question was not specific. Sandwiches were the most popular food choice for lunch, selected by 27.9 % of the participants. Rice was the second most popular lunch option (22.3 %). For dinner however, rice was the most popular food choice (38 %) followed by pasta (26.9 %) and last but not the least was the sandwich option (4 %).

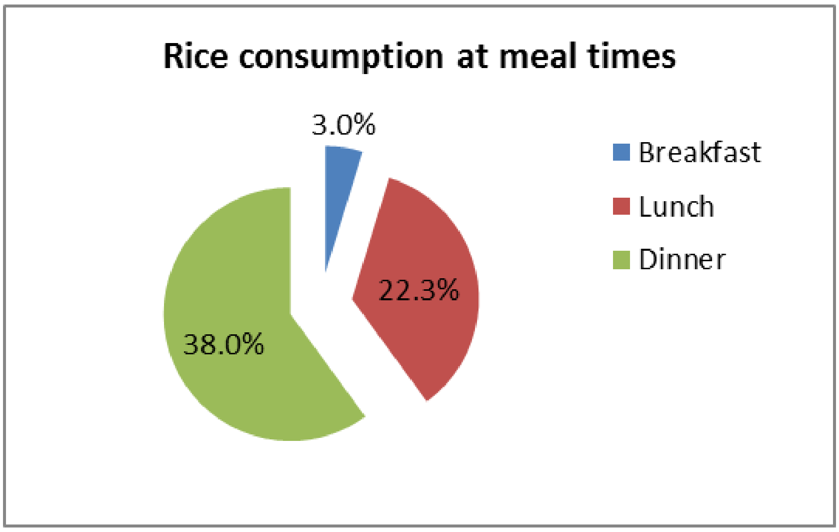


Figure 6.8. Rice consumption pattern at different times of the day.

6.5. Discussion

Rice is a staple food for more than half of the world's population, but recent studies have brought about the awareness that humans can be significantly exposed to inorganic As through consumption of rice and rice-based products (Stanton *et al*., 2015; Signes-Pastor *et al*., 2016; Davis *et al*., 2017).

According to the survey, majority of the White British consume a greater amount of rice per serving but at a lower frequency in comparison to the combined ethnic group in which the opposite was observed. Overall, the Bangladeshi community showed a higher frequency of rice consumption (more than once a day). These results are in line with previous literature which highlights that the Bangladeshi are the largest rice consumer population in the UK, consuming an average of 30 times more than the White British (Meharg, 2007).

The results obtained from this study reveal that over half of the participants (56.5 %) have basic knowledge about As, with more male participants showing greater knowledge of this carcinogen. We can also conclude that more of the older generation (45+) had basic knowledge of As in comparison to the other age groups; therefore age is definitely linked to general As knowledge. The relationship between social economic status and As knowledge revealed that level of education and occupation could potentially play a role in general knowledge of As. Education of up to 'secondary school' level and the 'volunteer' followed by 'employed' groups had high number of participants with As knowledge in comparison to the other groups. Similar results were observed in a study conducted by Ababio and Adi (2012) were participants educated up to tertiary level were more aware of foodborne diseases in comparison to the uneducated and those with only basic or secondary level of education.

Although over half of the participants had basic knowledge of As, only 14.5% knew about exposure to As from rice intake. Thereby, revealing that the issue of As contamination in rice is not publicised enough. Knowledge of As did not translate into safety practices related to rice preparation and consumption amongst the participants. Over half (57.3%) of the participants aware of As consumed rice more than twice a week. 84.7% did not rinse their rice before cooking and 57.5% used the 1:2 cooking method.

When questioned about their attitude towards rice preparation after the study, majority of those with prior knowledge of As admitted that they would stick to the usual way of preparing rice. However the opposite was observed amongst those without prior knowledge of As contamination in rice, with over half of them stating that they were willing to adopt cooking techniques that would reduce As content in rice. This is consistent with Kaptan *et al*. (2017), Pask and Rawlins (2016) and Taylor and Snyder (2017) who explain that people adopt protective behaviours when they perceive risks.

According to Rundmo and Nordfjaern (2017) the knowledge of the hazard/risk determines risk perception and behaviour. However, in this study, most participants did not associate As, which they perceive as toxic to health with rice consumption; hence they had no views on risk perception from As exposure through rice consumption. For example, a participant explained that “he knows that As is toxic because it is used to make rat poisons, hence it can be toxic to human health, however, he has never heard of people eating rice and having health issues due to As”, therefore he did not associate rice with As. Therefore, the lack of knowledge of As in rice results in low/no risk perception (Liu *et al*., 2014).

Nevertheless, some participants knew about As contamination in rice and yet they were not willing to adopt any protective behaviour. The plausible explanation for this could be due to the predisposition of people to believe that they are less vulnerable to particular risks whereas other people are more susceptible to such risks (van Dijk *et al*., 2011; Li *et al*., 2016). For instance, a participant from the combined group mentioned that “due to As regulations on food products here in Europe/UK, there is less possibility of As in rice and that it is more of an issue for people in endemic areas”. This opinion also suggests trust in safety regulation institutions (Kher *et al*., 2011; Li *et al*., 2016). Furthermore, the low score of knowledge of As in rice may be because the issue of health risks from As exposure in rice is not a significant health hazard in the UK (Kaptan *et al*., 2017) unlike elsewhere (Tonsor *et al*., 2011; Mitman, 2014).

The study also investigated As knowledge of different ethnic groups to gain an idea of the awareness of this contaminant amongst rice eating communities in Manchester. From the results obtained, we can report that the vast majority of the participants who knew about As originated from the White British group (92%). It was surprising that there was a lower knowledge of As amongst the other ethnicities even though many participants are originally from As endemic areas. However, this may be due to the fact that they were born in the UK or have been resident here for a very long time and therefore are unaware of the severity of As contamination in their countries of origin. Another factor that could contribute to the lower knowledge is that they might originate from areas/provinces where As is not endemic.

Results revealed that a higher percentage of Bangladeshis (53.8 %) were aware of As in rice in comparison to the other groups. However, regardless of this, they still had a high frequency of rice consumption amongst all the participants. On the other hand, no participants from the Pakistani or African/Caribbean groups had knowledge of As contamination in rice, which is worrisome, considering that the Pakistani group (36.1%) came second for the group that consumes a huge amount of rice (2 or more cups). Hence, lower to no knowledge of As amongst some ethnicities may suggest that they are more susceptible to As exposure through rice consumption, taking a cue from Hooper and Kolar (2017) which indicates that lower knowledge of e-cigarette among African American/Black and Hispanics smokers may lead to greater use of e-cigarette.

Attitudes of the participants with regards to rice preparation after the study revealed that majority of participants from the combined ethnicities were not willing to adopt new cooking techniques efficient in reducing As content in rice, in comparison to the White British group. Similarly, a higher proportion of the combined group were not willing to reduce frequency, quantity of rice or consider food options other than rice. All in all, the lack of desire in changing behaviour after the study could be attributed to low perception of risk associated with As exposure from rice consumption, due to rice being their staple food and part of their culture/tradition (Son et al., 2011; Jacobs *et al*., 2015)

Overall, majority of the participants perceived rice as beneficial to health. This could be because the participants are familiar with rice as a healthy product rather than as a source of risk (Jacobs *et al*., 2015). Additionally, Signes-Pastor *et al*. (2015) and Hite (2013) state that due to its nutritional content, palatability, aroma, lightness and low allergenic potential, rice is easily accepted. Furthermore, Jacobs *et al*. (2015) posits that familiarity decreases consumers' feelings of uncertainty and increases perceived control towards a food product and the benefits of a food product are more significant in the mind of a consumer than risks. For example, a participant mentioned that "she has been eating rice since she was a child and she has not had any health issues because of rice. Therefore, she does not think there is any health risk associated with rice consumption". Additionally, Ueland *et al*. (2012) posits that "perception of benefit is based on heuristics (easy decisions and simple intuitive strategies) whereas risk perception is based on cognitive or rational information processing". Therefore, this may further explain why most participants viewed rice as being beneficial than harmful.

In conclusion, results from the study reveal that although a higher proportion of the participants had general knowledge of As, very few were aware of As contamination in rice, probably due to the lack of association of As with rice. Prior knowledge of As in rice did not always result in the use of recommended practices involved in rice preparation and consumption. In addition, the White British were more favourably inclined to minimise As exposure from rice by reducing frequency and amount of rice consumption and considering other food options. Thus, suggesting that the other ethnicities perceive low to no risk whilst the White British may perceive risk of exposure to As from rice intake.

Chapter 7

A Systematic literature review on arsenic content in some popular cereal grains.

7.1. Abstract

Upon conducting the population survey on exposure to As through rice consumption, results presented in the previous chapter revealed that apart from rice, other grains consumed include wheat, maize/corn and oats. Hence, I decided to conduct a systematic literature review on As content of some of these grains. Barley and oats contained the lowest As concentration, below maximum limit of 0.5 mg/kg in China and 1 mg/kg for Australia and New Zealand, whilst maize and millet contained some of the highest As concentrations, possibly due to being grown on an industrial area. This was background research, hence, further investigation should be carried out on As contamination in maize and millet, especially in areas were As contamination in soil and groundwater is an issue.

7.2. Introduction

Cereal grains have been the fundamental component of the human diet for many years and have played a considerable role in shaping human civilisation (Awika, 2011). They provide carbohydrates, proteins, B vitamins and minerals for majority of the world’s population (McKevith, 2004). Cereals also provide about 60% of calories for populations in the developing world whilst 30% of calories in the developed world is derived from direct cereal consumption (Awika, 2011). An increase in the global cereal production has been observed over the years and production for 2018 stands at 2587 million tonnes (FAO, 2018). More specifically, according to the FOA (2018) there has been an increase in the harvest of wheat, maize and barley from Brazil and the Russian Federation.

Out of all the cereal grains, As contamination is more prominent in rice. Research has shown that transfer of As from soil to grain is an order of magnitude greater in rice than in wheat and barley (Ruttens *et al*., 2018). However, inspite of this, exposure to As from other cereals is possible where rice is not the staple as stated by Zhao *et al* (2010); ‘contribution of wheat to human intake of inorganic As is small for wheat crops grown in uncontaminated soils but becomes significant for those grown in soils with elevated As’.

In this study, a systematic literature review was conducted to investigate the As content of some popular cereal grains consumed around the world.

7.3. Methods

7.3.1. Search Strategy

An extensive systematic search was conducted in Web of Science, Pubmed and Scopus to retrieve literature on arsenic contamination in some popular grains, between the year 2000 and 2018. Search terms included arsenic, heavy metals, wheat, maize, barley, oats, rye, millet, sorghum, cereals, grains alone or in combination with ‘AND’, ‘OR’ or ‘IN’. The literature search and article retrieval were conducted according to the PRISMA guidelines (**Fig. 7.1**) (Moher et al., 2010).

7.3.2. Inclusion and exclusion criteria

The titles, abstracts and full texts were screened by one investigator. Following initial screening, all potentially eligible articles were downloaded. Inclusion criteria were:

- Full text available
- Articles in the English language
- Detected total As in either wheat, maize, barley, oats, rye, millet or sorghum grains
- Not a greenhouse or experimental study
- Published between 2000 and 2018.

Articles that did not meet the criteria above were excluded.

7.3.3. Data extraction

Data obtained from the selected articles was thoroughly checked and the data searched for included year or study (where applicable), year of publication, sample size, geographical region of study, mean concentration of As, method of detection and first author.

7.4. Results and discussion

7.4.1. Study characteristics

Out of 1167 articles that were reviewed, 50 fulfilled the inclusion criteria (**Fig. 7.1)**.The distribution is as follows; 19 articles reported on As in wheat, 17 on maize, 1 on oats, 3 on barley, 2 on rye, 4 on millet and 4 on sorghum.

The total number of samples from all the articles combined was 920, and the different methods of analysis reported were atomic absorption spectroscopy (AAS), atomic fluorescence spectroscopy (AFS), hydride generation atomic fluorescence spectrometry (HG-AFS), inductively coupled plasma mass spectrometry (ICP-MS), flow injection hydride generation atomic absorption spectrometry (FI-HG-AAS), high resolution inductively coupled plasma mass spectrometry (HR-ICP-MS),), inductively coupled plasma dynamic reaction cell mass spectrometry (ICP-DRC-MS), neutron activation analysis (NAA), flame atomic absorbtion spectroscopy (FAAS), inductively coupled plasma atomic emission spectroscopy (ICP-AES) and gamma-ray spectrometer (GRS). The mean As concentration of wheat, maize, barley, oats, rye, sorghum and millet was extracted from the 50 papers.

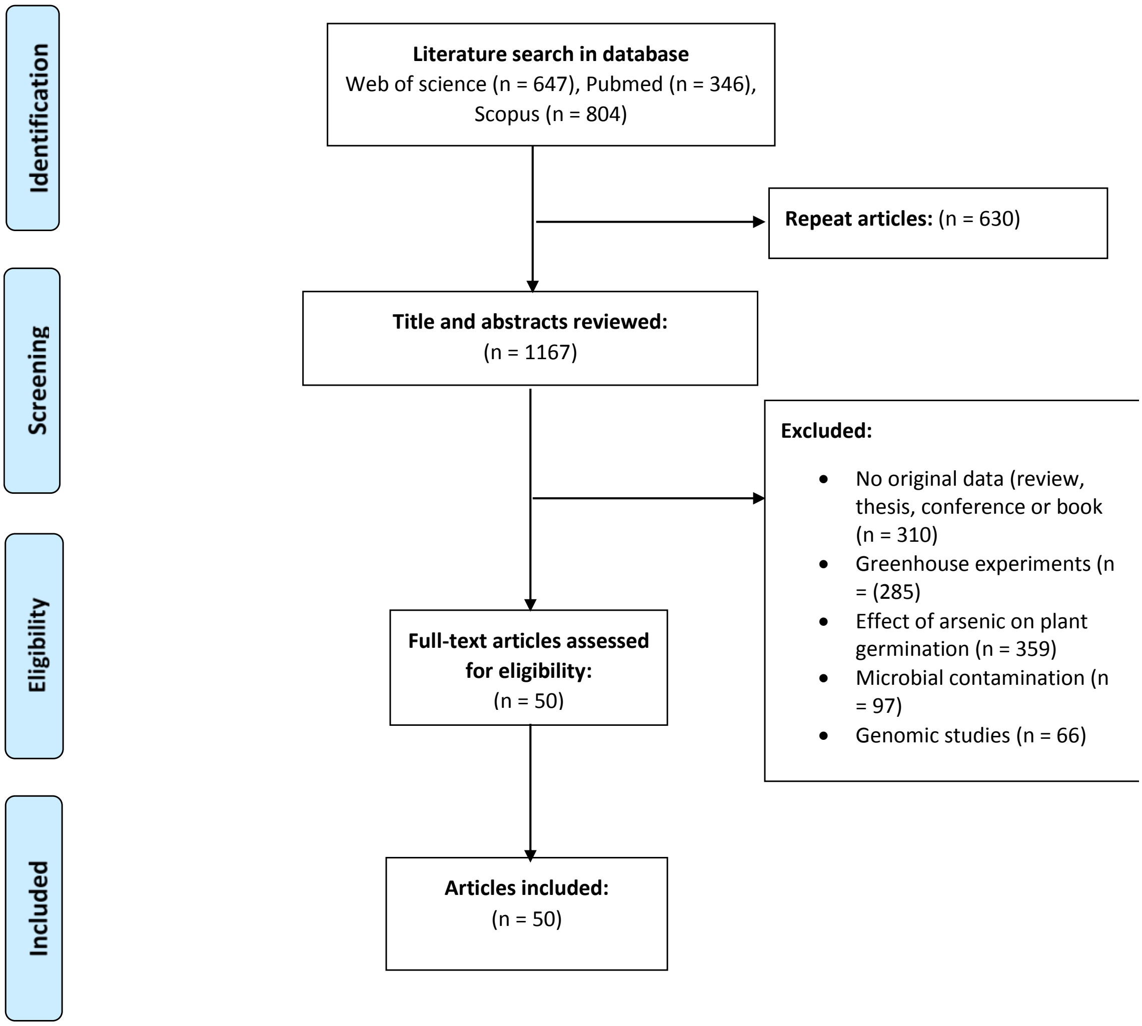


Figure 7.1. Flow diagram showing the search and selection process, following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines.

7.4.2. Arsenic concentration in wheat

According to Cubadda *et al*. (2010) and D'amato *et al*. (2011), wheat is an important source of inorganic As for populations reliant on a wheat based diet. However, processing and cooking methods could lead to a decrease in wheat As content (Cubadda *et al*., 2003). The range of As concentration in wheat in this study was 0.001 to 0.74 mg/kg (Mohamed *et al*., 2017; Norra *et al*., 2005). Comparison of As content of wheat from different countries revealed that wheat from Saudi Arabia contained the lowest mean As concentration followed by wheat from the USA, 0.001 and 0.013 mg/kg respectively (Mohamed *et al*., 2017; Punshon and Jackson, 2018). Both studies were based on samples sourced from the local markets in Najran city, Saudi Arabia and Hanover and West Lebanon, USA. On the other hand, Pakistan (0.324 mg/kg) and India (0.314 mg/kg) had the highest mean levels of As in wheat. The high levels of wheat from Pakistan could be attributed to the use of contaminated ground (Rasheed et al., 2018) and tube well water (Baig *et al*., 2011) for irrigation. Similarly, in India, irrigation with As contaminated water (Bhattacharya et al., 2010; Kumar et al., 2016; Norra *et al*., 2005; Roychowdhury *et al*., 2002) caused an increase in As content of wheat whilst high selenium rich soil reduced As in wheat (Skalnaya *et al*., 2018). In Adomako *et al*. (2011) study, the mean concentration of As in wheat originating from USA and China was 0.05 mg/kg. This value is lower in comparison to the mean (0.096 mg/kg) of As content in wheat from these particular countries, in the other studies considered in this review. The big sample size, sampling area - vicinity to a lead smelter (Xing *et al*., 2016) and coal mine (Shi *et al*., 2013) and the use of waste water for irrigation could account for the high As levels in wheat from the current study. The levels of As in wheat observed in this review were below the limit of 1mg/kg stipulated by the Food Standard Agency (FSA) of Australia and New Zealand (**Table 7.1**). A few samples from Pakistan and India exceeded the Chinese limit of 0.5 mg/kg As in cereals (Baig *et al*., 2011; Norra *et al*., 2005).

Table 7.1. Stipulated limit for As concentration in cereals

Country	Limit (mg/kg)
China	0.5
Australia and New Zealand	1.0

Table 7.2. Arsenic content of wheat

Author	**Year of publication**	**Sample origin**	**Sample size**	**Arsenic concentration (mg/kg)**	**Method of analysis**
Cubadda *et al.*	2010	Italy	141	Northern - 0.010, Central - 0.0082, Southern - 0.0083	ICP-MS
D'Amato *et al*.	2011	Italy	8	0.0298	ICP-MS
Roychowdhury *et al*.	2002	India	Jalangi block - 11, Domkal block - 23	Jalangi block - 0.219, Domkal block- 0.233	ICP-MS
Bhattacharya *et al.*	2010	India	8	0.129	
Shi *et al*.	2013	China	Area A - 45, Area B - 30	Area A- 0.0345, Area B - 0.0321	HG-AFS
Rasheed *et al*.	2018	Pakistan	8	0.105	ICP-DRC-MS
Zhang *et al*.	2018	China	Dongdagou stream – 22, Xidagou stream - 14	Dongdagou stream - 0.417, Xidagou stream - 0.224	AFS
Raber *et al*.	2012	Italy	1	0.165	ICP-MS
Punshon and Jackson	2018	USA	15	0.013	ICP-MS
Cubadda *et al*.	2003	Italy	3	Sample 1 -0.0084, S2 - 0.0102, S3 - 0.0074	
Adomako *et al*.	2011	Global	19	0.05	ICP-MS
Williams *et al*.	2007	Scotland	29	Scotland - 0.03	ICP-MS
Williams *et al*.	2007	England	37	0.07	ICP-MS
Baig *et al*.	2011	Pakistan	40	Faiz Ganj - 0.22, Thari Mirwah - 0.35 and Gamba - 0.62	AAS
Xing *et al*.	2016	China	25	0.183	AAS
Kumar *et al*.	2016	India	35	0.027	ICP-MS
Skalnaya *et al*.	2018	India	Nawanshahr-Hoshiarpur - 9, Patiala - 9	Nawanshahr-Hoshiarpur - 0.010, Patiala - 0.020	ICP-MS
Mohamed *et al*.	2017	Saudi Arabia	5	0.001	ICP-MS
Norra *et al*.	2005	India	3	Plant 1 - 0.71, Plant 2 - 0.74, Plant 3 - 0.74	ICP-MS
Bronkowska *et al*.	2008	Poland	12	0.056	AAS

7.4.3. Arsenic concentration in maize

Maize production is high in countries such as China, Argentina, Mexico and India. However, these areas are also known to have very high hot spots for soil As concentrations, thereby posing a great risk for As contamination in maize (Rosas-Castor *et al*., 2014). The range of As concentration in maize was 0.007 to 2.09 mg/kg (Skalnaya *et al*., 2018; Mishra *et al*., 2014) (**Table 7.3**). Korean and Malaysian maize contained similar As concentrations (0.04 mg/kg), as a result of man-made contamination of agriculture soil due to industrial, municipal waste and the use of phosphate fertilisers (Zarcinas *et al*., 2004) and copper-tungsten mining (Jung *et al*., 2002). Maize originating from India had the highest mean As concentration (0.649 mg/kg). Although Sharma et al. (2018) and Skalnaya *et al*. (2018) reported low average values of 0.06 mg/kg and 0.007 respectively, the average value of 1.59 mg/kg reported by Mishra *et al*. (2014) raised the total mean of As in maize from India. Maize investigated in Mishra's study was cultivated in Yamuna flood plain, an area known to have high levels of As in superficial water. As content in maize from Chile, China and Pakistan was within the range of 0.015 to 1.85 mg/kg (Munoz *et al*., 2002; Queirolo *et al*., 2000). Soil-plant As contamination in these countries was through geological processes (Munoz *et al*., 2002; Queirolo *et al*., 2000), mining and industrial activities (Aguilar *et al*., 2018; Liu *et al*., 2005; Wu *et al*., 2016) and use of contaminated water (Boashan *et al*., 2005; Neidhart *et al*., 2012; Baig *et al*., 2011; Husaini *et al*., 2011). Overall, some samples from China (1.48 mg/kg), Chile (1.85 mg/kg) and India (2.09 mg/kg) had mean As values exceeding both the Chinese (0.5 mg/kg) and the FSA (1 mg/kg) maximum limit of As in cereals.

Table 7.3. Arsenic content of maize

Author	Year of publication	Sample origin	Sample size	Arsenic concentration (mg/kg)	Method of analysis
Rosas-Castor *et al.*	2014	Mexico	MTA - 6	MTA 1- 0.32, 2- 0.23, 3- 0.27, 4- 0.24, 5- 0.32, 6- 0.33	HG-AFS
Sharma *et al.*	2018	India	5	0.06	FAAS
Adomako *et al.*	2011	Global	89	0.01	ICP-MS
Barac *et al.*	2015	Serbia	14	0.095	ICP-OES
Baig *et al.*	2011	Pakistan	55	Faiz Ganj- 0.19, Thari Mirwah- 0.25, Gambat- 0.39	AAS
Neidhardt *et al.*	2012	China	2	0.06	HR-ICP-MS
Skalnaya *et al.*	2018	India	Nawanshahr-Hoshiarpur -9, Patiala - 9	Nawanshahr-Hoshiarpur - 0.007, Patiala - 0.010	ICP-MS
Liu *et al.*	2005	China		SZY - 0.21, GYB - 1.48, JTC - 0.12	ICP-MS
Wu *et al.*	2016	China	20	0.13	ICP-MS
Baoshan *et al.*	2005	China	14	0.46	NAA
Queirolo *et al.*	2000	Chile		1.85	INAA
Aguilar *et al.*	2018	Chile		Control area - 0.03, Mining area - 0.06	AAS
Mishra *et al.*	2014	India	2	V11 - 2.09, V12- 1.08	AAS
Jung *et al.*	2002	Korea	3	0.04	ICP-AES
Husaini *et al.*	2011	Pakistan		Faisalabad - 0.43, Gujranwala - 0.47	GRS
Zarcinas *et al.*	2004	Malaysia	10	0.042	ICP-MS
Munoz *et al.*	2002	Chile		a - 0.404, b -0.015, c- 0.152	FI-HG-AAS

Table 7.4. Arsenic content of barley and oats

Cereal grain	Author	Year of publication	Sample origin	Sample size	Arsenic concentration (mg/kg)	Method of analysis
Barley	Williams *et al.*	2007	Scotland	6	0.04	ICP-MS
	Williams *et al.*	2007	England	29	0.08	ICP-MS
	Kim *et al.*	2008	Korea	Exposed area - 7, Control - 6	Exposed area - 0.005, Control - 0.007	ICP-MS
	Bronkowska *et al.*	2008	Poland	7	0.047	AAS
Oats	Sigrist *et al.*	2016	Argentina	4	0.018	FI-HGAAS

7.4.4. Barley and Oats

Barley is a widely adaptable crop because it can be grown in temperate areas as a summer crop and in tropical areas as a winter crop. The range of As concentration in these grains was 0.005 to 0.08 mg/kg (**Table 7.4**). Arsenic levels observed were all very low and below the maximum permissible limit stipulated by China and FSA of Australia and New Zealand for As in cereals (**Table 7.1**). In England, Scotland (William *et al*., 2007) and Korea (Kim *et al*., 2008) barley contained lower As levels in comparison to rice. The anaerobic growing conditions of rice make it more favourably inclined to take up more As in comparison to the aerobic conditions required to grow barley (Williams *et al*., 2007). Similarly, As content of oats purchased from supermarkets in Santa Fe, Argentina was ten times lower than that of polished rice (0.18 mg/kg) in Sigrist *et al*. (2016) study.

7.4.5. Rye, millet and sorghum

According to Adriano (1986), rye is a high-stress and As tolerant crop which is able to survive in infertile, acidic or sandy soils. The range of As concentration in rye was 0.115 to 0.52 mg/kg (**Table 7.5**). Rye from Spain (Álvarez-Ayuso *et al*., 2016), grown in an old mining area had the highest average As content (0.43 mg/kg) in comparison to rye from Poland (Bronkowska *et al*., 2008), originating from two copperworks regions. Although the mean concentration of 0.43 mg/kg is lower than the maximum limit, two rye samples from Spain contained As levels just above the Chinese limit (0.5 mg/kg).

The range of As concentration in millet was 0.01 to 3.31 mg/kg (Adomako *et al*., 2011; Brahman *et al*., 2014). Pakistan had the highest mean concentration (1.21 mg/kg) of As in millet in comparison to Ghana (Brahman *et al*., 2014; Husaini *et al*., 2011a; Husaini *et al*., 2011b). This level was above Chinese and FSA limits of 0.5 and 1 mg/kg respectively.

The range of As concentration in sorghum was 0.01 to 2.22 mg/kg (Adomako *et al*., 2011; Liu *et al*., 2005). The concentration of 2.22 mg/kg in sorghum grown on soil covered with mine tailings reported by Liu *et al*. (2005) was six times higher than the As concentration of sorghum grown on soil which had been cleared of mine tailings, in the same study. Thereby indicating that mine tailings are a great source of As contamination. Another study from China observed As levels of 0.5 mg/kg in sorghum that was grown in areas surrounding a municipal waste dump site (Liu *et al*., 2007). The mean value of As in sorghum (1.03 mg/kg) originating from

China was higher than that of Ghana and Pakistan (Adomako *et al*., 2011 and Baig *et al*., 2011) and above the maximum limit for As in cereals.

In conclusion, barley and oats contained the lowest As concentration (all below maximum limit of 0.5 mg/kg in China and 1 mg/kg for Australia and New Zealand) in comparison to the other grains whilst maize and millet contained some of the highest As concentrations, possibly due to being grown on an industrial area. Further investigation is required on As contamination in maize and millet, especially in areas were As contamination in soil and groundwater is an issue.

Table 7.5. Arsenic content of rye, millet and sorghum.

Cereal grain	Author	Year of publication	Sample origin	Sample size	Arsenic concentration	Method of analysis
Rye	Álvarez-Ayuso *et al.*	2016	Spain		75 m - 0.52, 125m - 0.52, 150m - 0.25	ICP-AES
	Bronkowska *et al.*	2008	Poland	3	0.115	AAS
Millet	Adomako *et al.*	2011	Ghana	9	0.01	ICP-MS
	Husaini *et al.*	2011	Pakistan		Faisalabad - 0.79, Gujranwala - 0.4	GRS
	Brahman *et al.*	2014	Pakistan	9	3.312	AAS
	Husaini *et al.*	2011	Pakistan		0.33	NAA
Sorghum	Adomako *et al.*	2011	Ghana	6	0.01	ICP-MS
	Baig *et al.*	2011	Pakistan	48	Faiz Ganj - 0.18, Thari Mirwah- 0.23, Gambat - 0.55	AAS
	Liu *et al.*	2007	China	3	0.5	ICP-MS
	Liu *et al.*	2005	China		GYB - 2.22, JTC - 0.38	ICPMS

Chapter 8

General discussion

8.1. Main findings

This thesis provides an insight into As contamination in rice at a laboratory and population level. Excess water rice cooking as a form of short term As mitigation technique has been studied by multiple researchers (Carey *et al*., 2015; Mihucz *et al*., 2007; Raab *et al*., 2009). However, very few studies (Gray *et al*., 2015; Mihucz *et al*., 2010) have focussed on the loss of essential nutrients as the adverse effect of this practice. The current study is the first of its kind to determine how essential element loss affects contribution to the recommended daily intake. Therefore, it is essential to fill this gap by conducting research that investigates the benefits as well as the risks associated with rice cooking techniques. The use of excess water rice cooking to reduce As content of rice is relevant both in the west and in other parts of the world where rice is a staple. However, the loss in essential nutrients mainly affects communities reliant on a rice-based diet and who are unable to afford other nutritious foods which can be used to substitute the loss due to rinsing and cooking of rice. The resultant effect is the increase in the risk of micronutrient deficiency (Harrison, 2011). Furthermore, exposure to As has been linked the development of CKDu in Sri Lanka. In order to contribute to limited data on As content in rice of Sri Lankan origin, a preliminary study was carried out to determine the As content of rice samples obtained from CKDu endemic areas in comparison to existing literature. This study produced a wide variation of As content in rice which could be attributed to sample size, temporal variation and market based compared to field based study. Based on this study, it is apparent that rice from CKDu endemic areas might have As. However, potential ecological risk of CKDu from As in rice needs further investigation. General knowledge of As amongst the White British and ethnic minority groups was high. However, very few participants were aware of As contamination in rice. Prior knowledge of As in rice did not always result in the use of recommended practices. In comparison to consumers from the ethnic minority groups, the White British were more favourably inclined to reduce the amount and frequency of rice consumed, and consider food options other than rice. Thus, suggesting that the other ethnicities have low to no risk perception of As exposure through rice consumption whilst the White British may perceive risk of exposure to As from rice.

Furthermore, results obtained from the survey revealed that apart from rice, other popular grains consumed include wheat, maize/corn and oats. This information formed the basis of the systematic literature review in chapter 7 and the results obtained showed that As contamination was higher (above 0.5 mg/kg limit for China and 1 mg/kg for Australia and New Zealand) in maize and millet in comparison to the other cereal grains.

8.2. Applications

The following highlight the practical contribution of the research presented in this thesis:

- Provide data on heavy metal contamination in rice which will contribute to achieving the Food Standards Agency's (FSA) main objective: protect public health from risks which may arise in connection with the consumption of food.
- Contribute towards one of the goals of the Food and Agriculture Organisation of the United Nations (FAO) to eradicate hunger, food insecurity and malnutrition by providing information on how excess water cooking can affect essential nutrients retained in rice and thereby influence their contribution to the RDI.
- Provide the first ever dataset on risk perception of As exposure through rice consumption in a population of a low risk country.
- To enhance research in the vital area of nutrition in ethnic minority populations in the UK. Research and interventions in this area are very poor according to the British Nutrition Foundation (BNF).
- Data collected from the questionnaire survey will provide unique information on the dietary habits of ethnic minority groups and this will help health professionals to tailor programmes that will benefit these groups in making healthier food choices.

8.3. Further recommendation

8.3.1. Laboratory work

Further analysis in the form of As speciation could be carried on the rice samples to determine the content of organic and inorganic As, in order to establish the safety of rice consumption. Additionally, a larger sample size and a variety of cooking techniques can be utilised in future studies. A study to establish a link between rice consumption and CKDu in Sri Lankan might include a large number of rice samples collected from CKDu endemic areas, in addition to biological samples like blood, urine, hair and nails, collected from CKDu patients.

8.3.2. Questionnaire survey

A future questionnaire survey should involve a larger sample size, more ethnic groups and should encompass a wider area of Manchester to give a better representation of the rice eating community. This survey can also be done on a country wide scale. A restaurant or takeaway survey can also be carried out to investigate rice cooking practices because if consumers are not cooking their own rice, they are purchasing it from restaurants or takeaways. In addition, a translator can also be used during the study for participants who cannot understand English, or the questionnaire could be translated into multiple languages to cater for non-English speakers.

8.3.3. Systematic literature review

A more in-depth review can be carried out on the As (total and speciation studies) content of other foods in the food chain, in different areas, especially those experiencing an issue of As contamination.

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Appendix

Supplementary data for **chapter 5**

Reference	**Sample type**	**size**	**Location**	**As (mg/kg)**
Jayasekera and Freitas (2005)	Market based		Raw rice - Producer 1	0.03
Jayasekera and Freitas (2005)	Market based		Raw rice - Producer 2	0.03
Jayasekera and Freitas (2005)	Market based		Parboiled rice - Producer 1	0.07
Jayasekera and Freitas (2005)	Market based		Parboiled rice - Producer 2	0.09
Chandrajith et al. (2011)	S1R1		Giradurukotte	0.14
Chandrajith et al. (2011)	S1R2		Giradurukotte	0.09
Chandrajith et al. (2011)	S1R3		Giradurukotte	0.11
Chandrajith et al. (2011)	S1R4		Giradurukotte	0.11
Chandrajith et al. (2011)	S1R5		Giradurukotte	0.10
Chandrajith et al. (2011)	S2R1		Nikawewa	0.19
Chandrajith et al. (2011)	S2R2		Nikawewa	0.16
Chandrajith et al. (2011)	S2R3		Nikawewa	0.12
Chandrajith et al. (2011)	S2R4		Nikawewa	0.16
Chandrajith et al. (2011)	S2R5		Nikawewa	0.26
Jayasumana et al. (2015)	New improved varieties - Field based	20	Padaviya	0.16
Jayasumana et al. (2015)	New improved varieties - Field based	17	Sripura	0.19
Jayasumana et al. (2015)	New improved varieties - Field based	25	Maha Wilachchiya	0.14
Jayasumana et al. (2015)	New improved varieties - Field based	17	Mihinthale	0.14
Jayasumana et al. (2015)	New improved varieties - Field based	19	Kurunegala	0.15

Jayasumana et al. (2015)	New improved varieties - Field based	11	Monaragala	0.13
Jayasumana et al. (2015)	New improved varieties - Field based	11	Gampaha	0.10
Jayasumana et al. (2015)	Traditional varieties - Field and market based	10	Kalu Heenati	0.03
Jayasumana et al. (2015)	Traditional varieties - Field and market based	10	Mada Thawalu	0.03
Jayasumana et al. (2015)	Traditional varieties - Field and market based	10	Pachcha Perumal	0.03
Diyabalanage et al. (2016)		81	Wet Zone	0.05
Diyabalanage et al. (2016)		70	Intermediate Zone	0.04
Diyabalanage et al. (2016)		75	Dry Zone	0.04
This study	Market based		Anuradhapura	0.05
This study	Market based		Anuradhapura	0.05
This study	Market based		Anuradhapura	0.03
This study	Market based		Anuradhapura	0.19
This study	Market based		Trincomalee	0.03
This study	Market based		Trincomalee	0.04
This study	Market based		Trincomalee	0.14
This study	Market based		Vavuniya	0.27
This study	Market based		Vavuniya	0.21
This study	Market based		Vavuniya	0.40
This study	Market based		Vavuniya	0.13

IS RICE SAFE? AN INVESTIGATION OF HOW ARSENIC CONTAMINATION AFFECTS THE NUTRITIONAL CONTENT OF RICE AND AN EXPLORATION OF THE PUBLIC PERCEPTION OF RICE SAFETY.

This questionnaire should only be completed by participants who have given informed consent to take part in the study. Participants are advised that they are not obliged to answer any questions they are not comfortable with.

Date:

Time:

Location:

RICE AND WATER ASSESSMENT

Rice Source and Intake

Type of rice consumed most frequently (1 or more options)	Source of rice	Frequency of Rice Consumption	Amount Consumed at one time per person (Grams or cups) *1 cup = 200g*
Basmati Long grain Brown Jasmine Regular/medium grain Wild Other	Cultivated Supermarket African/Asian Other	Never 1-6 times per year 7-11 times per year 1 time per month 2-3 times per month 1 time per week 2 times per week 3-4 times per week 5-6 times per week 1 time per day 2 or more times per day	< 1 cup 1 cup 2 cups > 2 cups

Rice accompaniment

What do you have with your rice most frequently?	Vegetables Meat Fish Milk Other

Rice cooking information

Do you wash your rice before cooking?	Amount of rice cooked at one time? (Per person) (Grams/cups)	What is the ratio of rice to water used in the cooking process?	How much water (if any) is discarded after rice has been cooked?	Do you use the same source of water to wash and cook your rice?	What is the source of this water?
Y N	<1 cup 1 cup 2 cups 3 cups 4 cups <4 cups	1:1 1:2 1:4-1:6 >1:6	None <1/4 1/4 1/2 >1/2	Y N	Tap water Supermarket

DIETARY INTAKE ASSESSMENT

Do you consume any of the rice based products listed below?

Which of these rice products do you consume the most? ***1 or more option***			**E.g Frequency of consumption**
None **Rice Krispies** **Rice Crackers** **Rice Cakes** **Rice Noodles** **Rice Flour** **Rice Drinks** **Rice Syrup** **Rice Vinegar** **Rice Bran Oil** **Rice Wine** **Other**	**Frequency**	**Quantity**	**Never** **1-6 times per year** **7-11 times per year** **1 time per month** **2-3 times per month** **1 time per week** **2 times per week** **3-4 times per week** **5-6 times per week** **1 time per day** **2 or more times per day**

Which of these grains do you normally eat? ***(1 or more options)***	**None** **Wheat** **Oats** **Corn** **Barley** **Rye** **Millet** **Sorghum** **Quinoa** **Lentils** **Other**
Which of these foods do you consume the most for Breakfast? ***(1 or more options)***	**Nothing** **Rice** **Cereals** **Bread** **Fruit** **Other**
Which of these foods do you consume the most for Lunch? ***(1 or more options)***	**Nothing** **Salad** **Sandwich** **Rice** **Pasta** **Potatoes (including chips)** **Other**
Which of these foods do you consume the most for Dinner? ***(1 or more options)***	**Nothing** **Salad** **Sandwich** **Rice** **Pasta** **Potatoes (including chips)** **Other**

OTHER QUESTIONS

Access to media	**Do you:** - **Own a television/radio Y N** - **Have access to the internet? Y N** - **How often do you listen/ watch the news?** **Never** **1-6 times per year** **7-11 times per year** **1 time per month** **2-3 times per month** **1 time per week** **2 times per week** **3-4 times per week** **5-6 times per week** **1 time per day** **2 or more times per day** - **How often do you read the newspaper?** **Never** **1-6 times per year** **7-11 times per year** **1 time per month** **2-3 times per month** **1 time per week** **2 times per week** **3-4 times per week** **5-6 times per week** **1 time per day** **2 or more times per day**
Knowledge on arsenic contamination	- **Have you ever heard of arsenic? Y N** - **If yes, how much do you know about it?** **Little** **Average** **A lot** - **Before this study, were you aware that some rice may contain arsenic? Y N** - **Do you believe that arsenic is toxic to the human health? Y N** - **Do you believe that it is possible for humans to be exposed to arsenic through rice consumption? Y N**
Nutritional Knowledge	- **Do you think rice is nutritious? Y N** - **If yes, what nutrients can you get from rice?** - **Do you believe that cooking rice in excess water can affect nutrients in rice? Y N** - **If yes, how? Open question**
Cooking practices	- **Have you inherited any cooking practices from your parents/other relatives Y N** - **Is your cooking influenced by your ethnic background? Y N** - **Before today, had your knowledge on arsenic in rice influenced your cooking practices? Y N** - **If yes, what did it change? Open question**

	- After today, will your knowledge on arsenic affect: How you cook your rice Y N If yes, what will change? Open question How much rice you will consume? Less The Same More How often you will consume rice? Less Often The Same More Often
Food options and expenditure	- Do you prefer meals cooked at home or takeaways? Home Takeaway - When you eat out or buy takeaways, what do you normally order? Pizza Pasta Rice Potatoes Including (chips) Salad Other - After today, will you consider other food options other than rice? Y N - How much do you spend on food per week? <£10 £10-£20 £20-£50 £60-£100 >£100

PERSONAL DETAILS

Name	
Gender	
Age	
Ethnicity	
Country of Origin	
Nationality	
Education **(Highest level attained)**	**1. Primary** **2. Secondary/ GCSEs** **3. Higher education (university)** **4. Other; Specify……………**
Occupation **(Please give details)**	**1. Self Employed** **2. Employed** **3. Unemployed** **4. Student** **5. Volunteer** **6. Other**
Housing	**1. House owner** **2. Tenant** **3. Other** **Time resident at current property** **0-3 months** ☐ **3-6 months** ☐ **6-12 months** ☐ **12 months +** ☐ **Time resident at previous property** **0-3 months** ☐ **3-6 months** ☐ **6-12 months** ☐ **12 months +** ☐

University of Salford MANCHESTER

Consent Form

Is Rice Safe? An investigation of how arsenic contamination affects the nutritional content of rice and an exploration of the public perception of rice safety.

Please tick the appropriate boxes	**Yes**	**No**
I have read and understood the project information sheet dated …/…/…..	☐	☐
I have been given the opportunity to ask questions about the project.	☐	☐
I agree to take part in the project. Taking part in the project will involve me answering a questionnaire, including a Food Frequency Assessment.	☐	☐
I understand that my taking part is voluntary; I can withdraw from the study at any time and I do not have to give any reasons for why I no longer want to take part.	☐	☐

Use of the information I provide for this project only

I understand my personal details will not be revealed to people outside the project.	☐	☐
I understand that my words may be quoted in publications, reports, web pages and other research outputs.	☐	☐

Please choose one of the following two options:

- I would like my real name to be used in publications. ☐
- I would not like my real name to be used in publications. ☐

Use of the information I provide beyond this project

I agree for the data I provide to be archived at the University of Salford.	☐	☐
I understand that other genuine researchers will have access to this data only if they agree to preserve the confidentiality of the information as requested in this form.	☐	☐
I understand that other genuine researchers may use my words in publications, reports, web pages, and other research outputs, only if they agree to preserve the confidentiality of the information as requested in this form.	☐	☐

____________________________	__________________________ _	__________________
Name of participant [printed]	**Signature**	**Date**
______________________________	__________ __________ ______	__________________
Researcher [printed]	**Signature**	**Date**

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