NON-CANCER RISKS AND MITIGATION STRATEGIES OF Fe, Mn, Cu and Cr IN MILLED MAIZE FLOUR

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DECLARATION

I, Denis Ainebyona (18/U/GMFT/22179/PD) declare that this work is original and has never been submitted to any university or other higher institution of learning for a similar or other academic award.

Signature……………………………………   Date…………………………………….

APPROVAL

This is to certify that the work presented herein this dissertation is the student's own, done by him under our supervision, and is now ready for submission for the award of the Degree of Master of Science in Food Technology of Kyambogo University.

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DEDICATION

To the almighty God, My family and friends for the prayers, love and support towards my life and education.

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LIST OF ACRONYMS AND ABBREVATIONS

AACC	American Association of Cereal Chemists
AAS	Atomic Absorption Spectrophotometer
ADD	Average Daily Dose
ADI	Acceptable Daily Intake
CDI	Chronic Daily Intake
CODEX	Codex Alimentarius Commssion for Food Standards
CSF	Cancer Slope Factor
FAO	Food Agriculture Organization
HM	Heavy Metal
HQ	Hazard Quotient
HI	Hazard Index
HR	Health Risks
ILCR	Incremental Lifetime Cancer Risk
IPCS	International Programme on Chemical Safety
KCCA	Kampala Capital City Authority
LOAEL	Lowest Observed Adverse Effect Level
MAAIF	Ministry of Agriculture, Animal Industry and Fisheries
MCDA	Multi Criteria Decision Analysis
MTIC	Ministry of Trade, Industry and Cooperatives
NOAEL	No observed Adverse Effect Level
RfD	Oral reference dose
USEPA	United States Environmental Protection Agency

ABSTRACT

In Uganda, maize (*Zea mays*), is milled into flour that is used as an ingredient in many food products including baby foods. Milling is mainly done in hammer mills that are fabricated using mild steel. Mild steel is made up of iron (Fe), manganese (Mn), copper (Cu) and chromium (Cr) as the major heavy metals (HM). During milling, hammer mill parts wear out and release metal particles into the flour. Heavy metals are a risk factor for chronic illnesses such as cancer, diabetes and heart disease. The safety of maize flour with regard to HM contamination arising from milling operations in Uganda is not known. This study aimed assessed the non-cancer risk and mitigation strategies to minimize Fe, Cu, Cr and Mn exposure in maize flour. A total of fifty samples (25 maize grain and 25 maize flour) were obtained from 5 milling enterprises in Kampala city. Metal concentration was determined using Atomic Absorption Spectrophotometry (AAS). Non-cancer risks due to HM exposure were determined using the non-cancer hazard quotient (HQ) and hazard index (HI) described by the United States Environmental Protection Agency (US EPA). Multi-Criteria Decision Analysis (MCDA) using Promethee-Gaia software 1.4 Academic Edition was used to determine the best risk management option. Data was analyzed with A General Linear model using SPSS for Windows, Version 16.0 (2007), SPSS Inc, Chicago, IL, USA. Milled flour samples had significantly ($p < 0.05$) higher concentrations of HM than the grain. Heavy metal concentration ranged from 0.257 to 1.782, 0.016 to 0.198, 0.122 to 0.501 and not detected (ND) to 1.151 mg/kg for Fe, Cu, Cr and Mn, respectively. The HQ and HI values were less than the United States Environment Protection Agency (US EPA) management level of 1 for both children and adults. Consumers of maize flour produced in hammer mills in Kampala will not experience adverse health effects. Nevertheless, the bioaccumulation of HM in the body organs poses a danger. Therefore, the possible development of risk should be monitored on a regular basis with the view of putting in place measures to protect public health.

CHAPTER 1: INTRODUCTION

1.1 Background

Maize (*Zea mays* L.) is the second most produced crop in the world after sugar cane (Santpoort, 2020). Maize is a staple food for large populations in Latin America, Africa, and Asia, where it is consumed as "corn on the cob" and used to prepare various kinds of traditional foods. The United States (US), China and Brazil are the top three maize-producing countries in the world, producing *ca.* 563 million metric tons/year (Ranum, Pena-Rosas, & Garcia-Casal, 2014). Africa contributes *ca.* 7.4% towards the global production (717 metric tonnes) of maize with South Africa and Nigeria as the leading producers (FAOSTAT, 2020). Uganda is ranked the 8th largest producer in Africa and 3rd in East Africa (Daly, Hamrick, Gereff, & Guinn, 2016). The country's production in 2017 was estimated at 2.7 million tonnes after Tanzania and Kenya at 5.6 and 3.4 million tonnes, respectively (FAOSTAT, 2020). Maize contains approximately 72% starch, 10% protein, and 4% fat, supplying an energy density of 365 Kcal/100 g (Ranum *et al.*, 2014; Ranum, 2016). Per capita maize consumption in Uganda ranges from 28 to 125 kg per annum (Buteme, Masanza, & Bwayo, 2020). Maize is third most important food in terms of caloric intake after plantains and cassava (Haggblade & Dewina, 2010). The maize is used as a raw material in the production of food and animal feeds, and industrial products such as starch, sweeteners, vegetable oil and alcohol (Orhun & Ebru, 2013; Shah, Prasad, & Kumar, 2016). Cereal flours have improved palatability and bio-availability of nutrients when compared to the whole grain (Oghbaei & Prakash, 2016). Uganda is Africa's second-leading exporter of maize flour (Daly, Hamrick, Gereff, & Guinn, 2016). Maize flour is produced through a dry milling process mainly by small and medium scale (hammer) millers (Axtell, Fellows, Ged, Lubin, & Musoke, 2004). According to Yadav, Abbas, & Patel (2014), hammer mills are fabricated using mild steel that comprises Fe (99.2%), Mn (0.404%), Cr (0.16%) and Cu (0.009%).

Iron, Mn, Cr and Cu are heavy metals (HM) that constitute a major public health problem, and food consumption is a major pathway for exposed humans (Odusote, Soliu, Ahmed, Abdulkareem, & Akande, 2017; Issa, Yasin, Loutfy, & Ahmed, 2018). The HM are naturally occurring non-degradable elements that have a high atomic weight and a density greater than 5 gcm^{-3} (Tchounwou, Yedjou, Patlolla, & Sutton, 2012). Metals such as lead (Pb) and Chromium(VI) (Cr(VI)) are poisons to all living organisms and at very low concentrations (Shafiuddin *et al*., 2019). They are non-biodegradable and cumulative poisons that are potentially toxic. Manganese (Mn) is an endocrine-disrupting chemical (Tchounwou, Yedjou, Patlolla, & Sutton, 2012). Their actions can lead to adverse developmental, reproductive, neurological, immunological, teratogenic and carcinogenic effects in mammals. The main sources of HM in the environment include emissions from industries, industrial effluents, use of leaded gasoline and paints, agricultural activities, indiscriminate disposal of municipal wastes and incineration of toxic substances (Muwanga & Barifaijo, 2006). Heavy metal release as a result of wear and tear from milling machines also contributes substantially to the total contamination of foods (Hajeb, Sloth, Shakibazadeh, Mahyudin, & Afsah-Hejri, 2014; Odusote, Soliu, Ahmed, Abdulkareem, & Akande, 2017). Iron (Fe), Mn, Cu and Cr are major components of mild steel (Kalagbor *et al*., 2017).

Contamination of food with HM is a key issue, with serious repercussions on public health (Issa, Yasin, Loutfy, & Ahmed, 2018). However, information on the health risks of HM exposures in milled products is limited (Liang *et al*., 2019). Public health risk assessment provides a flexible platform for planners to estimate the risks of public health emergencies in their jurisdiction (Peters, Hipp, Kricun, & Cherna, 2019). Risk is the chance of harmful effects to human health or to ecological systems resulting from exposure to a stressor, which may be a chemical such as HM.

It is the combination of the probability that a hazard may occur in a food product and the effect of exposure to the hazard on human health (Van-der-Fels-Klerxa *et al*., 2018). Risks are classified as either cancer or non-cancer. Cancer risks follow the linear no-threshold model (LNT) that is used to estimate the health risk. Non-cancer risks on the other hand, exhibit a threshold value below which no adverse effects are observed. This study aimed to assess the levels of HM in maize flour and the associated non-cancer risks to consumers.

1.2 Problem statement

Food safety is one of the major problems currently facing the world and hindering food security efforts such as increased food production, processing, storage and distribution to final consumers (Mieke, Eelco, & Oliver, 2016). Maize has safety associated challenges such as aflatoxin contamination which together with HM contamination could render maize flour a potential health risk to consumers. Grinding machines in Uganda use mild steel grinding discs for size reduction. The hammers are fabricated with little or no quality assurance to ascertain the critical engineering properties hence resulting into poor wear resistance (Normanyo, Esiam, & Amankwa-Poku, 2010; Ngabea, Okonkwo, & Liberty, 2015). The wear and tear of the contact metal surface during grinding produces fine metal particles in the maize flour. Bioaccumulation of HM in the human body due to consumption of contaminated maize meal over prolonged period of time elicits chronic health effects, and is also a risk factor for cancers such as cancer of the liver and lungs (Tchounwou *et al*., 2012; Carver & Gallicchio, 2018). Information on the safety of maize flour in regard to HM contamination arising from milling operations is limited.

1.3 Justification of the study

The Sustainable Development Goals (SDG 2, 3 and 9 emphasize promotion of inclusive and sustainable industrialization while reducing the number of deaths and illnesses from hazardous chemicals. Maize is commercialized globally and the safety of maize-based products is of international interest (Bordini *et al.*, 2019). Studies on HM contamination have largely centered on environmental pollution deriving from emissions of industries, industrial effluents, use of paints, agricultural activities, indiscriminate disposal of municipal wastes and incineration of toxic waste (Muwanga & Barifaijo, 2006). There is insufficient information on food contamination with HM resulting from milling machinery and equipment in Uganda. It was therefore anticipated that the results obtained from the study, would serve as a baseline to highlight the effect of milling equipment on the levels of HM in milled foods. In addition, the results would help to promote both local and international trade.

1.4 Objectives of the study

1.4.1 General objective

To assess the non-cancer risk and mitigation strategies of Fe, Cu, Cr and Mn in hammer milled maize flour consumed in Kampala city.

1.4.2 Specific objectives

The specific objectives of the study were to:

1. Determine the levels of Fe, Mn, Cu and Cr in the dry maize grain and the resulting maize flour.
2. Estimate the Fe, Mn, Cr and Cu exposure and non-cancer risks for adults and children in Kampala city.

3. Determine the best mitigation strategy to reduce HM contamination in hammer milled maize flour.

1.5 Research questions

1. Does milling maize in hammer mills raise the levels of Fe, Mn, Cu and Cr?
2. Does consumption of maize meal prepared from milled flour processed in hammer mills pose a health risk to consumers due to heavy metal exposures?
3. What mitigation strategy would best reduce HM contamination in hammer milled maize flour?

CHAPTER 2: LITERATURE REVIEW

2.1 Maize

2.1.1 Taxonomy

Maize (*Zea mays* L.) also known as corn, belongs to the family Poaceae and genus Zea. The genus Zea consists of 4 species of which *Zea mays* is the only economically important spp (Shah, Prasad, & Kumar, 2016). The other Zea species, referred to as teosintes, are largely wild grasses native to Mexico and Central America. The number of chromosomes in *Zea mays* is 2n = 20 (Shah, Prasad, & Kumar, 2016). There are about 50 different varieties of maize grown throughout the world, and classification can be done on the basis of kernel shape, size, color, taste, etc. There are two major kernel shapes: round (flint maize) or tooth shape (dent maize). White, yellow and red are the most common colors for maize kernels, but varieties with red-brown, light red, pale yellow, orange and black kernels also exist. Most of the maize grown in the US is yellow, whereas people in Africa, Central America, and the southern US prefer white maize (Ranum *et al*., 2016). Yellow maize is not popular in Africa because: (1) yellow maize is associated with food-aid programs and therefore perceived as being consumed only by poor people, (2) yellow maize is associated with animal feed and (3) yellow maize is too sweet.

Maize varieties can be classified into 5 major groups according to specific food, feed and production needs: sweet corn, popcorn, flour corn, dent or field corn, and flint corn (Brown & Darrah, 1985; Verheye, 2010; Ranum, Pena-Rosas, & Garcia-Casal, 2014). Sweet corn is harvested prematurely, before the conversion from sugar to starch takes place. The sweet corn kernels therefore have more sugar than starch. Popcorn is also a variety that is used for human consumption. The popcorn kernel has a tough outer shell which encapsulates a small amount of soft starch content (Brown & Darrah, 1985). Flour corn is made of soft kernels consisting of soft starch content. This maize is easy to grind and is very often used in baked goods. Dent or field corn accounts for approximately 99% of all corn produced in the US. Dent corn is much starchier than sweet corn and therefore has a bland flavor and a mealy texture. Flint corn, which has a hard and glassy outer shell, is mainly grown in Central and South America for food and feed. White dent and flint varieties are commonly grown in developing countries, while yellow maize is more commonly grown in the rest of the world (Ranum, Pena-Rosas, & Garcia-Casal, 2014).

2.1.2 World production of maize

Maize *(Zea mays L.)*, is grown in many countries around the world with United States, China and Brazil as the top three leading producers. The total global production of maize in 2019 was estimated at 1148 million metric tons per year (Erenstein, Chamberlin, & Sonder, 2021). In Africa, South Africa is the leading producer with a total production of 11.3 million metric tons per year (FAOSTAT, 2020). Tanzania is the leading producer of maize in East Africa with a total production of 5.6 million tons, followed by Kenya at 3.9 million tons and Uganda at 2.6 million tons (Table 1). The Maize crop was introduced in Uganda around 1861 and is now grown in more than 50 districts (Babel & Turyatunga, 2015). It is cultivated by 1 to 5 million farmers, on about 1.5 million hectares of land and is a growing source of household income and foreign exchange (Erenstein, Chamberlin, & Sonder, 2021). Eastern Uganda accounts for 47% of total maize production, while Western, Central and Northern Uganda account for 21, 19 and 13%, respectively (Daly, Hamrick, Gereff, & Guinn, 2016).

Table 1. Maize production (metric tons) of selected African countries compared with the World leading maize producers for the period 2015 to 2019.

World/country	2015	2016	2017	2018	2019
World	1052608663	1127351276	1138653968	1124721882	1148487291
United States	345486340	412262180	371096030	364262150	347047570
China	265157307	263777750	259256299	257348659	260957662
Brazil	85283074	64188314	97910658	82366531	10113817
Africa	74036895	73818863	89301924	82896881	81891311
South Africa	9955000	7778500	16820000	12510000	11275500
Tanzania	5902776	6149000	6680758	6273151	5652005
Kenya	3825000	3339000	3186000	4013777	3897000
Uganda	2812917	2482795	2631728	2772718	2575000
Rwanda	370140	374267	358417	410280	421218
Burundi	160713	243740	228355	290498	270813
South Sudan	14000	107000	92000	90000	103000

Source: FAOSTAT (2020)

2.1.3 Maize utilization

Sixty five percent of the total world maize is used as livestock fodder and feeds production, 15% is used for food and 20% is used for ethanol and other industrial products production (Wilsner, Anderson, Plain, Hofstrand, & O'Brien, 2012). While the majority of the product in the developed world is used for industrial purposes and animal feed, maize is a staple food crop throughout much of Africa (Zilic, Milasinovic, Terzic, Barac, & Ignjatovic-Micic, 2011). Maize for human consumption is increasing mainly in developing countries, especially those in sub-Saharan Africa where populations are growing rapidly and white maize is an important staple for several countries. It is considered to be an important food source in countries where daily consumption exceeds 50 grams per person (Ranum, Pena-Rosas, & Garcia-Casal, 2014). The maize consumption level in Africa is between 52 to 328 g/per person/day (Ranum, Rosas, Pena, Casal, & Garcia, 2016). Besides, many African countries, such as South Africa, Uganda, Tanzania, Rwanda and Namibia, are important exporters of maize flour (Daly, Hamrick, Gereffi, & Guinn, 2016). About 28% of the maize grown in Uganda ends up in the domestic industry and is processed into flour, used for animal feed, and as a raw material for the beer industry (Daly, Hamrick, Gereffi, & Guinn, 2016).

2.1.4 Nutritional composition of maize

According to Sule, Umoh, Whong, Abdullahi, & Alabi (2014), maize contains moisture (11.6 to 20.0%), ash (1.10 to 2.95%), protein (4.50 to 9.87%), fat (2.17 to 4.43), fiber (2.10 to 26.70%) and carbohydrate (44.60 to 69.60%). In addition, maize is source of phytochemicals such as carotenoids, phenolic compounds and phytosterols that play an important role in preventing chronic diseases (Shah *et al*., 2016). Maize germ contains 45 to 50% oil that is used in cooking and salads. The oil contains 14% saturated fatty acids, 30% monounsaturated fatty acids, and 56% polyunsaturated fatty acids (Shah *et al*., 2016). Maize also contains important B complex vitamins, vitamin C and folic acid, and is a rich source of phosphorus (P), magnesium (Mg), Mn, zinc (Zn), Cu, Fe and selenium (Se). It is a good source of dietary fiber, low in fat and sodium (Shah *et al*., 2016). However, maize is naturally deficient in lysine and tryptophan, which are essential for humans.

2.1.5 Maize milling

Maize milling is a mechanical process that consists of several unit operations (Odusote, Soliu, Ahmed, Abdulkareem, & Akande, 2017). The process starts by the cleaning of the maize and ends with grinding and sieving into flour. There are two major technologies used for processing maize; dry and wet milling technologies (Gwirtz & Casal, 2014; Odusote, Soliu, Ahmed, Abdulkareem, & Akande, 2017). Each of the processing methods produces a finished product with a different nutritional composition and unique associated costs. The majority of the maize processors in Uganda use the hammer mill technology (Daly, Hamrick, Gereff, & Guinn, 2016). Hammer mills do not require a lot of capital investment and high skilled labour force to operate.

2.1.5.1 Dry milling process

In dry milling, corn is dry-fractionated into grits (endosperm), germ, pericarp fiber and flour (Anderson & Almeida, 2019). Industrial dry milling includes particle size reduction of clean whole maize with or without screening separation, retaining all or some of the original maize germ and fiber. Because of the high-fat content, these whole or partially degerminated maize products are not particularly shelf-stable (Gwirtz & Casal, 2014). Degermination of maize involves mechanical separation and processing, resulting in dry shelf-stable products with a majority of both germ and fiber removed. Much of the particle size reduction and separation is accomplished with equipment similar to that employed in wheat flour milling, including hammer mills, stone mills, roller mills, screeners, sifters, specific gravity separators, and aspirators (Gwirtz & Casal, 2014). Specialized equipment, such as degerminators and de-hullers or peelers, may be employed in maize processing. The conventional dry-milling process consists of a tempering-degerming milling process (Figure 1).

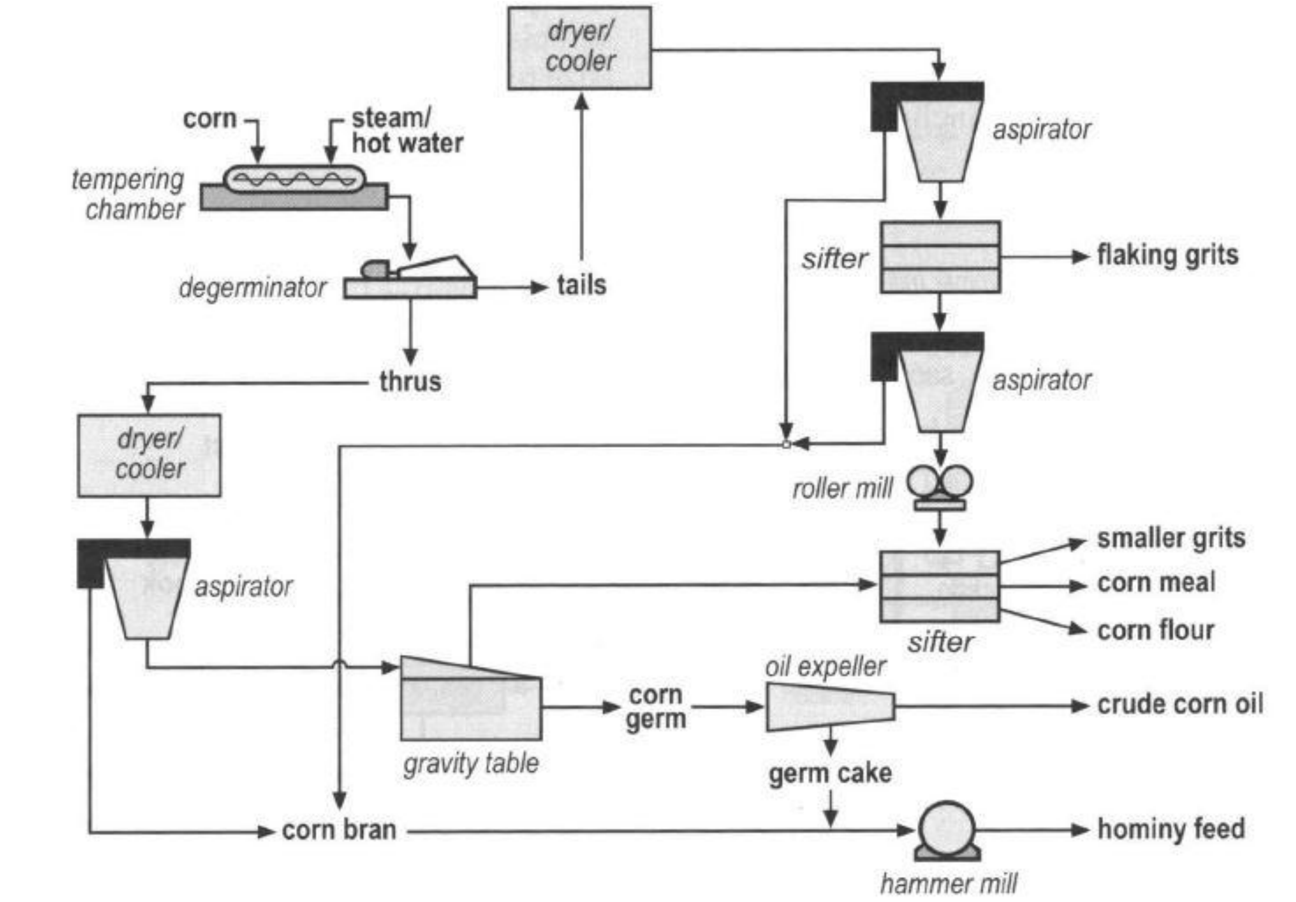

Figure 1: Maize dry-milling process schematic

Source: Rausch & Belyea (2006)

Tempering facilitates the removal of germ and bran (pericarp). Degerming produces two fractions ('tails' and 'thrus'). Tails are large pieces of endosperm (grits) that exit the tail end of the degerminator (Rausch & Belyea, 2006). Thrus consist of germ, bran and smaller endosperm pieces. Both the tails and thrus fractions are subjected to drying, cooling, aspiration, density separation and sizing to produce grits (flaking and other coarse grits). Grits are used for producing mainly breakfast cereals. Dry milled germ cake, bran and flour (standard meal) and broken corn are mixed together, dried and ground to make animal feeds. The hammer mill, a major technology in the dry milling process, is the one mainly used for milling grains to produce flour for human consumption, and bran used for animal feeds (Mugabi, Byaruhanga, Eskridge, & Weller, 2019).

2.1.5.2 Wet milling process

Wet milling is a larger, more versatile process used to produce a greater variety of products such as starch, corn syrup, sweeteners and ethanol (Daly, Hamrick, Gereff, & Guinn, 2016). In the wet-milling process, corn is fractionated into individual components of starch, protein, fiber, germ and solubles in an aqueous medium (Anderson & Almeida, 2019). Cleaned corn is conveyed to steep tanks, where it is hydrated counter-currently in 0.1 to 0.2% sulphur dioxide at 48° to 52°C for 24 to 36 hours. After steeping, corn is passed through attrition mills, which tear open the kernels and release the germ (Rausch & Belyea, 2006). Germ is recovered by density separation using hydro cyclones. The remaining slurry is passed through a set of screens to recover fiber and to wash residual starch and protein from fiber (Rausch & Belyea, 2006). Starch is washed to remove residual protein and is further processed to produce pearl starch, ethanol, corn syrups or other fermentation products. Protein, fiber and solubles are mixed together to produce corn gluten feed, which is used as an ingredient in ruminant animal diets. Wet-milled germ is used for recovery of corn oil, which is mainly used as human food. Figure 2 is a schematic representation of the maize wet-milling process.

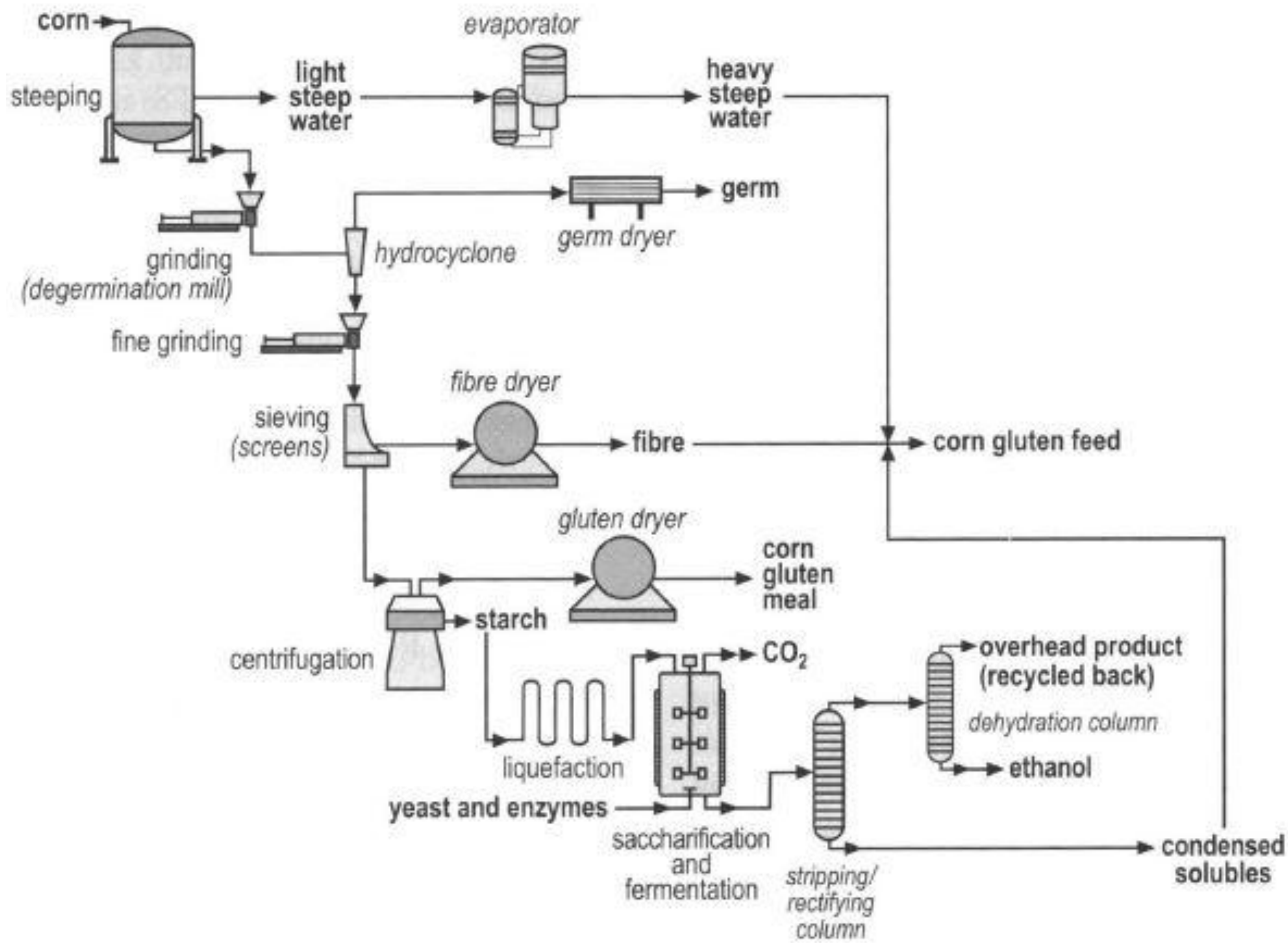

Figure 2: Maize wet-milling process schematic
Source: Rausch & Belyea (2006)

2.1.6 Hammer mill technology

A hammer mill is a crusher that can grind, pulverize, and crush a wide range of materials (Dabbour, Bahnasawy, Ali, & El-Haddad, 2019). It is simple in construction and the component parts can be easily replaced (Figure 3). Hammer mills are mostly impact grinders with swinging or stationary steel bars forcing ingredients against a circular screen or solid serrated section designated as a striking plate. The power requirement of the hammer mill is as low as 2.25kW 3hp (Kawuyo, Chineke, Ahmad, & Amune, 2014).

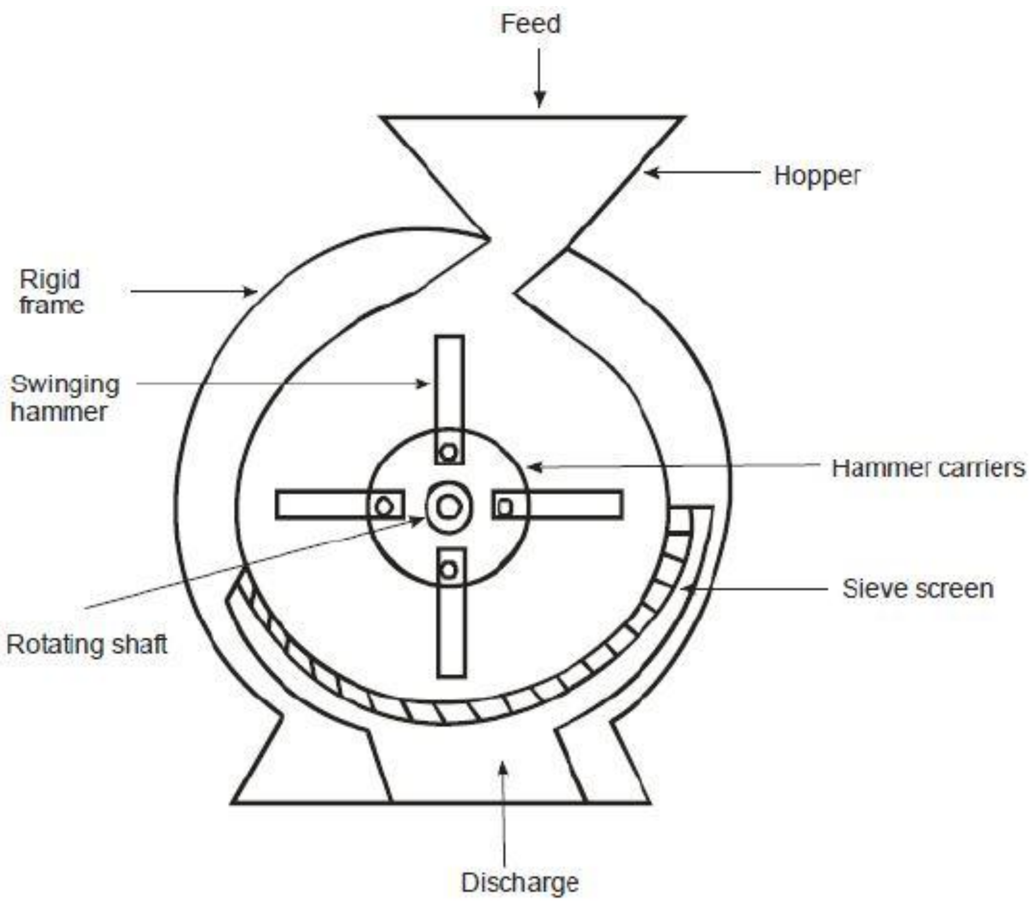

Figure 3: Diagrammatic representation of conventional hammer mill
Source: Kawuyo, Chineke, Ahmad, & Amune (2014); Dabbour, Bahnasawy, Ali, & El-Haddad (2019).

In the hammer mill, the maize grain is reduced in size to pass through the screen before it is discharged from the milling chamber. The material is held in the grinding chamber until it is reduced to the size of the openings in the screen. Size reduction occurs principally by impact and pulverization as the grain hits the hammers, the metal of the screen, and the back wall and front casing of the mill (Dabbour, Bahnasawy, Ali, & El-Haddad, 2019). The output of ground material varies according to the capacity of the motor, size of the perforations in the screen and variety and moisture content of the maize. The number of hammers on a rotating shaft, their size, arrangement and sharpness, the speed of rotation, wear patterns, and clearance at the tip relative to the screen or striking plate are important variables in grinding capacity and the appearance of the product (Kawuyo, Chineke, Ahmad, & Amune, 2014; Dabbour, Bahnasawy, Ali, & El-Haddad, 2019).

2.2 Heavy metals

Heavy metals are elements that have a density at least 5 times greater than that of water; specific gravity greater than 5 (Tchounwou, Yedjou, Patlolla, & Sutton, 2012). They also include metalloids that are able to induce toxicity at a low level of exposure. While metals such as Fe, Mn, Cu, Cr and Zn are required in the diet to maintain biochemical and physiological functions in humans, excess levels can create health risks (Raia, Lee, Zhang, Tsang, & Kim, 2019). The multiple industrial, domestic, agricultural, medical and technological applications of HM have led to their wide distribution in the environment; raising concerns over their potential effects on human health (Tchounwou, Yedjou, Patlolla, & Sutton, 2012). Heavy metals may enter the human body through food, water, air or absorption through the skin when they come in contact with humans. However, food is the major route of exposure to HM for non-occupationally exposed populations (Luigi Vimercati *et al*., 2016).

2.2.1 Sources of heavy metal

Heavy metals in food derive mainly from the environment. Reported sources of HM in the environment include; industrial, agricultural, pharmaceutical, domestic effluents, and atmospheric sources (Tchounwou, Yedjou, Patlolla, & Sutton, 2012). Industrial sources include metal processing in refineries, coal burning in power plants, petroleum combustion, nuclear power stations and high tension lines, plastics, textiles, microelectronics, wood preservation and paper processing plants. Environmental contamination can also occur through metal corrosion, atmospheric deposition, soil erosion of metal ions and leaching of HM, sediment re-suspension and metal evaporation from water resources to soil and ground water (Tchounwou, Yedjou, Patlolla, & Sutton, 2012). Among the agricultural sources, fertilizers, pesticides, and sewage sludge are the most common (Alengebawy, Abdelkhalek, Qureshi, & Wang, 2021). These sources significantly lead to the elevation of HM concentration and pollution in the ecosystem, e.g., smelting that results in releasing Cu, Zn, and As; insecticides that contribute to As release; burning of fossil fuels that produces Hg, and cars exhaust that releases Pb (Alengebawy, Abdelkhalek, Qureshi, & Wang, 2021). Processing equipment such as hammer mills, and materials used in packaging, have also been reported to be a source of HM contamination in food (Kalagbor, Fyneface, Korfii, Ogaji, & Kpoonanyie, 2017). Table 2 shows the elemental composition (%w/w) of mild steel.

Table 2: Elemental composition of mild steel (% w/w)

Metal	Fe	C	Si	Mn	P	S	Cr	Al	Cu
Composition (% w/w)	99.2	0.134	0.074	0.404	0.056	0.022	0.16	0.002	0.009

Source: Yadav, Abbas, & Patel (2014)

2.2.2 Heavy metal toxicity

Heavy metals are endocrine disrupting chemicals (EDC). An endocrine-disrupting chemical as defined by the U.S. Environmental Protection Agency (EPA) is an exogenous agent that interferes with synthesis, secretion, transport, metabolism, binding action, or elimination of natural blood-borne hormones that are present in the body and are responsible for homeostasis, reproduction, and developmental processes (Diamanti-Kandarakis *et al.*, 2009). Public health concern has been expressed about the potential role of EDC in increasing trends in obesity and diabetes, the major life-threatening diseases of the modern world. All hormone sensitive physiological systems are vulnerable to EDC, including brain and hypothalamic neuroendocrine systems; pituitary; thyroid; cardiovascular system; mammary gland; adipose tissue; pancreas; ovary and uterus in females; and testes and prostate in males (Diamanti-Kandarakis *et al*., 2009). Therefore, high doses of HM in the body have long term health effects (Kalagbor, Fyneface, Korfii, Ogaji, & Kpoonanyie, 2017).

2.2.2.1 Iron

Iron is the most abundant trace mineral in the body and is an essential element in most biological systems. The average adult stores about 1 to 3 g of Fe in the body with an intake of 8 to 18 mg/day (Abbaspour, Hurrell, & Kelishadi, 2014). It occurs in food in three forms; oxides, inorganic and organic salts, and organic complexes. The toxicity of Fe is governed by its bioavailability and is toxic in excess amounts. Problems that may result from iron toxicity include; anorexia, oligura, diarrhea, hypothermia, diphasic shock, metabolic acidosis, and gastrointestinal tract congestion (Normanyo, 2010). Iron is also a leading cause of unintentional poisoning deaths in children less than 6 years old. For Fe from all sources except for Fe oxides used as coloring agent, the established upper limit as a precaution against excessive Fe storage in the body is 45 mg per day (Khayat, Fanaei, & Abdolhakim, 2017). Excessive Fe in pregnancy can lead to adverse pregnancy outcomes including low birth weight, maternal hypertensive disorders, thrombotic risk and gestational diabetes through increased oxidative stress associated with increased insulin resistance (Parisi, Bartolo, Savasi, & Cetin, 2019). In addition, Fe has been suggested as a risk factor for colon inflammatory signaling and colorectal carcinogenesis through the loss of the key intestinal tumor suppressor gene Adenomatous polyposis coli (APC) located on the human chromosome 5q21 (Khayat, Fanaei, & Abdolhakim, 2017; Parisi, Bartolo, Savasi, & Cetin, 2019). However, in humans, except in idiopathic hemochromatosis, acute toxicity of Fe ingested from normal dietary sources has not been reported. Hemochromatosis refers to conditions, genetic or acquired, in which there is a systematic over accumulation of iron in the blood (Eid, Arab, & Greenwood, 2017; Milman, 2021). The excess free iron is taken up by tissues especially the liver, endocrine gland and the heart.

2.2.2.2 Manganese

Mn is the twelfth most abundant element in the earth's crust and is naturally present in rocks, soil, and water (Bouabid, Tinakoua, Lakhdar-Ghazal, & Benazzouz, 2016). Inorganic Mn is used in steel production, manufacture of dry cell batteries, production of potassium permanganate, manufacture of glass, leather and textile bleaching, matches and fireworks, oxidizing agent for electrode coating in welding rods. Organic compounds of Mn are used as fuel additive methylcyclopentadienyl manganese tricarbonyl, fungicides and as contrast agents in magnetic resonance imaging; MRI (Bouabid, Tinakoua, Lakhdar-Ghazal, & Benazzouz, 2016). It is an essential micronutrient with recommended intake of 1.8 to 2.3 mg but with potential toxicity at doses higher than 11 mg per day (Harischandra *et al*., 2019). Manganese is a powerful neurotoxin that causes learning disabilities and deficits in intellectual function in children and manganism and Mn-induced Parkinsonism in adults as well as compulsive behaviors, emotional lability, hallucinations, and attention disorders (Lucchini *et al*., 2017). Human overexposure to Mn through inhalation, ingestion or parental administration to a concentration of 75 mg/dl in the blood results in manganism. Manganism is a neurological condition that shows motor symptoms similar to Parkinson's disease (Harischandra *et al.*, 2019). High levels of Mn exposure in children may also produce undesirable effects on brain development and decrease in the ability to learn and remember (Lucchini *et al*., 2017).

2.2.2.3 Copper

Copper is an essential metal found in metalloproteins since evolutionary times (Bansal & Asthana, 2018). The recommended dietary allowance (RDA) for Cu is 0.9 mg/day for both male and female adults. However, Cu is toxic if consumed in excess of 10 mg/day (Bansal & Asthana, 2018). The primary toxic effects of Cu manifest in the liver as this is the organ where Cu accumulates after entering circulation. However, cancer resulting from a series of molecular events that change the normal cell properties is one of the most studied illnesses caused by Cu toxicity. Huge amounts of Cu have been associated with respiratory and urinary tract cancers (Baharvand *et al*., 2014).

2.2.2.4 Chromium

Chromium is the most abundant element in the earth's crust and is a typical transition element that forms many compounds that are colored and paramagnetic (Shekhawat, Chatterjee, & Joshi, 2015). The element forms compounds in the oxidation states of -2, -1, 0, +1, +2, +3, +4, +5, +6; the highest oxidation state, +6, corresponds to the sum of the numbers of 3d and 4s electrons. The oxidation states of -2, -1, 0 and +1 are formal oxidation states displayed by chromium in compounds such as carbonyls, nitrosyls and organometallic complexes (Shekhawat, Chatterjee, & Joshi, 2015). In the environment, Cr is mostly stable in the trivalent (+3) and hexavalent (+6) forms. The valence state of Cr is important because it controls the geochemistry and toxicity of the element. Trivalent Cr is essential in the metabolism of carbohydrates, lipids, and proteins mainly by increasing the efficiency of insulin (Lewicki *et al*., 2014). The adequate intake of Cr has been proposed as 35 and 25 µg/kg/day for men and women, respectively (Lewicki *et al*., 2014). Nevertheless, Cr can lead to life threatening complications when ingested in high amounts (Jagannati, Ramya, & Sathyendra, 2016). Hexavalent Cr-containing compounds are strong oxidants which act as human carcinogens, mutagens and teratogens in biological systems

(Beyersmann & Hartwig, 2008). Human exposure to airborne Cr^{6+} is associated with increased risk of lung cancer among workers in Cr-based industries. Chromium(VI) also causes increased risk of bone, prostate, hematopoietic system, lymphomas, Hodgkin's, leukemia, stomach, renal, and urinary bladder cancer (Welling, Beaumont, Petersen, Alexeeff, & Steinmaus, 2015).

2.3 Risk analysis

Risk is a function of the likelihood of occurrence of a hazard in food, and the severity of the adverse health effect on human health upon exposure to that hazard (Barlow *et al*., 2015). Risk analysis seeks to evaluate the impact of hazards on public health, identify appropriate mitigation strategies and maintain an ongoing transparent exchange of reliable information among stakeholders as a shared responsibility, to prevent the occurrence and subsequent human exposure to the hazards (Van der Fels-Klerxa *et al*., 2018). There are three distinct but connected components of food safety risk analysis; risk assessment, risk management and risk communication (Lindqvist, Langerholc, Ranta, Hirvonen, & Sand, 2020) as shown in Figure 4.

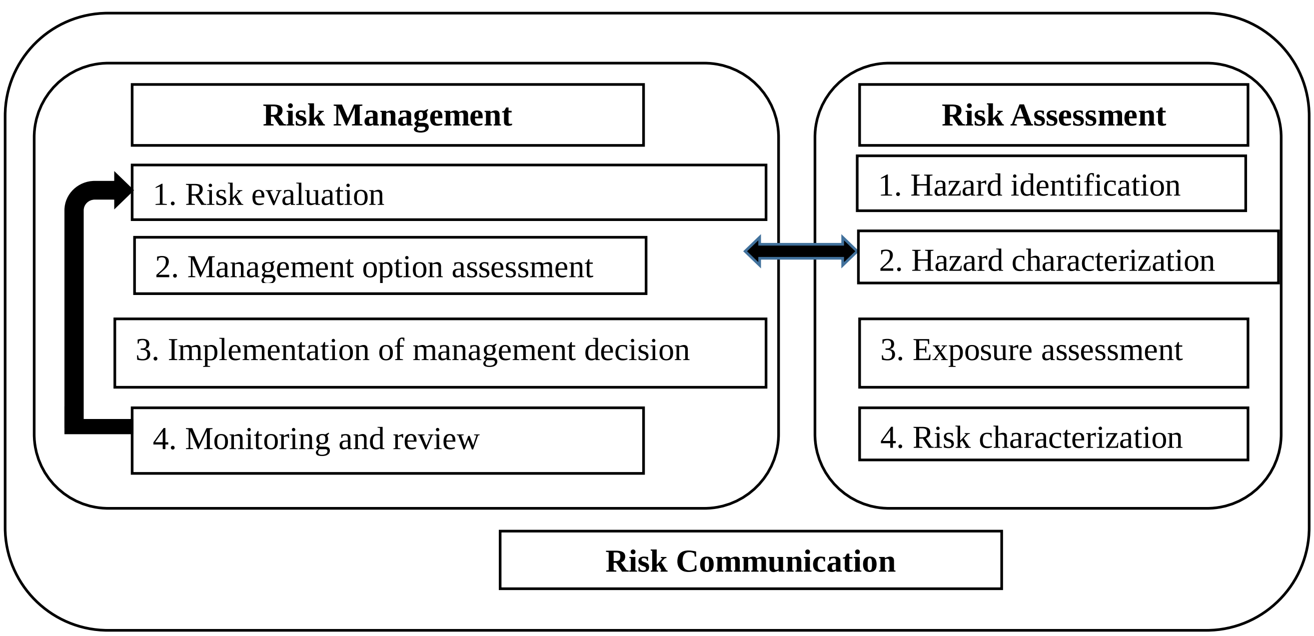

Figure 4: The food safety risk analysis framework.
Source: Wu (2012)

2.3.1 Risk assessment

Risk assessment is considered the central component of food safety risk analysis (Barlow *et al*., 2015). It provides the scientific basis for the establishment, implementation and continuous improvement of appropriate preventive and control measures for the risk management and risk communication processes (Van der Fels-Klerxa *et al.*, 2018). Risk assessment employs tools of science, engineering and statistics to identify and measure a hazard, determine possible routes of exposure, and finally use that information to calculate a numerical value to represent the potential risk. The process consists of four basic steps; hazard identification, hazard characterization, exposure assessment and risk characterization (Barlow *et al*., 2015; Grout *et al*., 2018; Chartres, Bero, & Norris, 2019).

2.3.1.1 Hazard identification

The first step in risk assessment is hazard identification, which examines whether a hazard has the potential to cause harm to humans and/or ecological systems at any level of exposure. Hazard identification focuses on identifying biological, chemical and physical agents capable of causing adverse health effects and which may be present in a particular food or group of foods (Van-der-Fels-Klerxa *et al.*, 2018). It requires determination of the known or potential adverse health effects associated with a particular hazard, through an in-depth review of available scientific information regarding the nature, occurrence, and mechanisms of action of hazards that influence their toxicity. The validity of the risk assessment process depends on the adequacy of the hazard identification step. It is important to ensure that declared hazard-food associations are realistic, both on the basis of empirical data and from experience on food production, processing, handling and consumption practices (Barlow *et al*., 2015). In general, hazard identification captures information including but not limited to characteristics of the hazard and the food in which they occur and how these characteristics favor their association, estimates of the concentration and prevalence of the hazard in the food, influence of food processing and/or preparation on the occurrence of the hazards, evidence of causal link between hazard and adverse health effect in humans.

2.3.1.2 Hazard characterization

Hazard characterization is a qualitative and/or quantitative evaluation of the nature of the adverse health effects associated with the hazard (Barlow *et al*., 2015). It is a description of the relationship between the levels of a hazard ingested in food (dose) and the probability and severity of its adverse health effects (Lindqvist, Langerholc, Ranta, Hirvonen, & Sand, 2020). Due to the variability in vulnerability in human populations, dose-response relationships differ for different sub-populations. Differences in age, gender, and genetics affect susceptibility of individuals to illness, and may thus require different dose-response relationships to describe the adverse health effects that may result from exposure (Lindqvist, Langerholc, Ranta, Hirvonen, & Sand, 2020).

Two models of dose-response relationships; threshold model and non-threshold model, have been described (Van-der-Fels-Klerxa *et al*., 2018). The threshold model assumes that there is a hazard dose level up to which some consumers can be exposed without suffering adverse health effects, while the non-threshold model assumes that all hazard doses can potentially cause an adverse health effect. In as far as chemical hazards are concerned, non-carcinogenic chemicals generally follow the threshold-model, whereas carcinogens follow the non-threshold model (Lindqvist, Langerholc, Ranta, Hirvonen, & Sand, 2020). For the threshold dose-response model, the thresholds considered are the lowest observed-adverse-effect level (LOAEL) and the no observed-adverse-effect level (NOAEL). The LOAEL is the lowest amount of a given hazard that causes a measurable adverse effect, while the NOAEL is the highest amount of the hazard which produces no measureable adverse effect in the most sensitive experimental subject (Lewis *et al*., 2002; Mao, Song, Sui, Cao, & Liu, 2019). Based on the NOAEL, health based guidance values such as acceptable daily intake (ADI) and tolerable daily intake (TDI) can be calculated for non-carcinogenic compounds (Mao, Song, Sui, Cao, & Liu, 2019).

2.3.1.3 Exposure assessment

Exposure assessment is the qualitative and/or quantitative evaluation of the likely intake of biological, chemical or physical agents via food as well as other sources and routes (Lindqvist, Langerholc, Ranta, Hirvonen, & Sand, 2020). It measures how much of a given hazard is likely to be ingested through food by a given population or a population subgroup (Barlow *et al*., 2015). Human dietary exposure assessments consider different durations of the exposure based on the outcome of the hazard characterization i.e., acute and/or chronic hazards (Lindqvist, Langerholc, Ranta, Hirvonen, & Sand, 2020). Chronic exposure estimates are based on individual average consumption over a survey duration whereas for acute exposure intakes per day are typically estimated (Ioannidou, Cascio, & Gilsenan, 2021). Chronic food consumption measures the average food consumption of at least two non-consecutive reporting days for a given food or group of foods and can be presented as grams per day. Dietary exposure is estimated by combining food consumption data for the total population or for the exposed sub-group who are the consumers, with food chemical concentration data (Ingenbleek *et al*., 2020; Ioannidou, Cascio, & Gilsenan, 2021). It can be estimated for single chemicals or multiple chemicals with the same mode of action or target organ (cumulative exposure). Chronic dietary exposure assessments performed by the joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Joint Meeting on Pesticide Residues (JMPR) most often use a simple deterministic model in which food consumption data are combined with data on the concentration of the chemicals in food (Arcella *et al*., 2019). To estimate the dietary exposure to hazards over long periods, chronic food consumption is combined with mean or median occurrence data to perform dietary exposure assessment. The resulting exposure estimate is compared with health-based guidance values for the chemical or microbiological agent of concern. Combining food consumption with chemical occurrence data forms the basis to calculate food safety indicators (Ingenbleek *et al*., 2020). Contrariwise, food safety indicators based on acute food consumption can be used to quantify potential exposure to biological or chemical hazards during a short period in time.

2.3.1.4 Risk characterization

Risk characterization is the qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterization and exposure assessment (Barlow *et al.*, 2015). It is the integration of the outputs of the previous steps to arrive at a qualitative statement of risk (e.g. low, medium, high) or a quantitative estimate (e.g. number of cancer incidents per year within the (sub-population of interest), including a description of the uncertainties and variability inherent in the entire risk assessment exercise (Lindqvist, Langerholc, Ranta, Hirvonen, & Sand, 2020).

2.3.2 Types of risk assessment

Two major types of risk assessments have been identified: chemical risk assessment, focusing on chemical hazards, and microbiological risk assessment that focuses on microbiological hazards (Lindqvist, Langerholc, Ranta, Hirvonen, & Sand, 2020). The steps discussed in sections 2.3.1.1 to 2.3.1.4 apply in both types of risk assessment. Based on the desired risk output, risk assessment can be qualitative (describes categories of risk as low, medium or high), quantitative (provides numerical risk estimates), or the semi-quantitative that evaluates risks using scores. The most applied procedure in microbial and chemical risk assessment, is the semi-quantitative procedure that utilizes a 4×4 risk matrix to define the risk level (EFSA, 2016). The risk matrix is a table used for allocating risk rankings for identified risks based on the probability or likelihood that a hazard will occur in the end product if control measures are failing or are completely absent, and the severity or effect of the hazard related to human health. Ranking of hazards is important because it enables risk-based priority-setting to support efficient resource allocation and utilization (Van der Fels-Klerx *et al*., 2018). Risk levels run on a scale of 1 to 7 (Table 3) and provide the basis for prioritization of the type of risk and action.

Table 3: The 4×4 risk matrix

Probability	High	4	4	5	6	7
	Real	3	3	4	5	6
	Small	2	2	3	4	5
	very small	1	1	2	3	4
			1	2	3	4
			Limited	Moderate	Serious	Very serious
			Effect			

Source: EFSA (2016)

Both the probability and effect are ranked on a 1 to 4 point scale. A probability of 1 (very small), has the theoretical chance that the hazard has never occurred before or there is a step in the production process that will eliminate or reduce the hazard to an acceptable level (EFSA, 2016). Alternatively, the level of product contamination is very low. A probability of 2 (small), means that there is likely to be failure or absence of prerequisite programs (PRP), and the occurrence of the hazard in the end product is very limited. Probability 3 (real), means that the failure or lack of a control measure does not result in the systematic presence of the hazard in the end product. However, the hazard can be present in a certain percentage of the end product in the associated batch. Finally, a probability of 4 (high), implies that the failure or absence of a control measure results in systematic error hence a high probability that the hazard will be present in all end products of the associated batch (AFSA, 2016).

In as far as effect or severity is concerned, 1 (limited), means that there will be no problem to the consumer related to food safety, or the hazard can never reach a dangerous concentration (EFSA, 2016). An effect of 2 (moderate), suggests no serious injuries and/or symptoms except when exposed to extremely high concentration for a long period of time. The hazard could have a temporary but clear effect on health. Effect 3 (serious), indicates a clear effect on health with short-term or long-term symptoms that rarely result in mortality (EFSA, 2016). Alternatively, the hazard has a long-term effect or the maximum safe dose is not known. Finally, an effect of 4 (very serious), means that the consumer group belongs to a risk category and the hazard can result in mortality. The hazard could also result in serious symptoms that can lead to permanent injuries or even mortality.

The risk level (R) is calculated as the product of the probability (P) and the effect (E) to the exposed population (EFSA, 2016; Van der Fels-Klerxa *et al.*, 2018). Heavy metals have been proven to pose long term health effects to humans (Thielecke & Nugent, 2018). Therefore, this study, considered the probability that HM will be in the final maize meal due to hammer milling to be real i.e., 3 and the health risk due to HM is moderate i.e., 2. The risk level is then obtained as 3

× 2 = 4, which implies moderate risk. A moderate risk (R = 3 or 4) can be acceptable, but the development of risk must be monitored on a regular basis with the view of putting in place protective measures (EFSA, 2016). A risk level of 1 or 2 is low, meaning acceptable risk but threats must be observed to discover changes that could increase the risk. On the other hand, a risk level of 5 to 7 is high and unacceptable hence remediation strategies must be put in place immediately (EFSA, 2016).

2.3.3 Risk management

Risk management is the process of weighing policy alternatives in consultation with all interested parties, considering risk assessment and other factors relevant for health protection of consumers and for promotion of fair trade practices and for selecting appropriate prevention and control measures (Attrey, 2017). It is the political arm of risk analysis that translates the results of risk assessments into actions, guided by governance priorities (IPCS, 2009). The risk management process should be transparent, consistent, and fully documented (Attrey, 2017). The process comprises four steps namely; risk evaluation (preliminary risk management activities), assessing management options, implementation of management decision, and monitoring and review. Risk evaluation determines the scope of risk assessments and also evaluates their outcomes. It includes the activities: identification of a food safety problem; establishment of a risk profile; ranking of the hazard and risk management priority; establishment of risk assessment policy; commissioning of risk assessment; and consideration of the result of risk assessment. The assessment of risk management options covers identification of the available management options, selection of a preferred option, evaluation of the impact of the preferred option on other factors (e.g. economic, social, and political impact), and a confirmation of the final decision. It is a difficult task to judge clearly, which of the management options is the best in a given case. Multi-Criteria Decision

Analysis (MCDA) methods are used to evaluate different alternatives based on multiple criteria using systematic analyses which overcome the limitations of unstructured individual or group decision-making (Bystrzanowska & Tobiszewski, 2018). Following implementation of the final decision, it is continually monitored and reviewed to measure its effectiveness to safeguard public health.

2.3.4 Risk communication

Risk communication is the interactive exchange of information and opinions throughout the risk analysis process concerning risk, risk-related factors and risk perceptions, among risk assessors, risk managers, consumers, industry, the academia and other interested parties, including the explanation of risk assessment findings and the basis of risk management decisions (Attrey, 2017; CAC, 2018). The aim of risk communication is to promote understanding and dialogue among stakeholders about decisions concerning the management of food safety risks, and to help consumers make informed judgments about the food safety hazards and risks (Maxim *et al*., 2021). Risk communication strengthens the effectiveness of risk management programs by equipping consumers with useful information about the risks associated with a food product to enable them to use or consume it safely; increasing public awareness of the nature of a food safety risk, and providing fair, accurate and appropriate information to enable consumers make informed choices on what suits their risk tolerance (Maxim *et al*., 2021). According to Attrey (2017), risk communication should: 1. promote awareness and understanding of specific issues under consideration during the risk analysis; 2. promote consistency and transparency in formulating risk management options/ recommendations; 3. provide a sound basis for understanding risk management decisions proposed; 4. improve overall effectiveness and efficiency of risk analysis; 5. strengthen the working relationships among participants; 6. foster public understanding of the process, so as to enhance trust and confidence in safety of food supply; 7. promote appropriate involvement of all interested parties; and 8. exchange information in relation to concerns of interested parties about risks associated with food. The purpose is to build stakeholder trust in risk managers and their work/decisions (Attrey, 2017).

2.3.5 Methods of determining health risks

Risk analysis and assessment methodologies include deterministic and probabilistic approaches (Marhavilas & Koulouriotis, 2012; Amirah, Afiza, Faizal, Nurliyana, & Laili, 2013). The deterministic or ‘‘point estimate’’ approach, consists of assigning a single representative value to each exposure parameter that appears in a risk equation (Rivera-Velasquez, Fallico, Guerra, & Straface, 2013). The approach is based on the selection of a fixed level in the distribution of consumption multiplied by a fixed value, chosen from the distribution of concentration usually obtained by field sampling and measurement. The fixed levels utilized to calculate a ‘‘point estimate’’ are generally chosen assuming a conservative scenario (Dorne *et al*., 2011).

Probabilistic risk assessment on the other hand utilizes methods such as the *Monte Carlo* simulation to derive a distribution of risk based on multiple sets of values sampled for random variables (EPA, 1999). In the probabilistic approach, each parameter in the risk equation is assigned a probability density function that describes the behavior of the risk in probabilistic terms. Thus, probabilistic risk analysis may provide more information than the deterministic approach (Rivera-Velasquez, Fallico, Guerra, & Straface, 2013). However, deterministic methods of risk assessment are often considered most appropriate for screening purposes. Therefore, the USEPA deterministic based risk models were be used in the proposed study.

2.3.6 Multiple-Criteria Decision Analysis for food safety decision making

Multi-Criteria Decision Analysis (MCDA) has been used in food safety to prioritize foodborne hazards, in the inspection of egg farms for monitoring compliance, and to guide the selection of food safety interventions (Ruzante, Grieger, Woodward, Lambertini, & Kowalcyk, 2017). Keeney and Raiffa define MCDA as an extension of decision theory that covers any decision with multiple objectives, while Belton and Stewart define it as an umbrella term to describe a collection of formal approaches that seek to take explicit account of multiple criteria in helping individuals or groups explore decisions that matter (Thokala *et al.*, 2016). The MCDA provides a framework for breaking down a complex decision into more manageable components, defining and understanding the relationship between the components (Drake, Hart, Monleón, Toro, & Valentim, 2017). The overall goal of MCDA methods is to evaluate alternatives based on multiple criteria using systematic analyses which overcome the limitations of unstructured individual or group decision-making. Hereby, MCDA improves the decision making process by: integrating objective values with subjective judgments; managing the decision-making process; and promoting transparency.

Multi-Criteria Decision Analysis utilizes a number of modeling approaches (Thokala *et al.*, 2016). Outranking methods involve making pairwise comparison of alternatives on each criterion, which are then combined to obtain a measure of support for each alternative being judged (Ruzante, Grieger, Woodward, Lambertini, & Kowalcyk, 2017). The outranking algorithms include the Elimination and Choice Expressing Reality (ELECTRE) family of methods, Preference Ranking Organization Method for Enrichment of Evaluations (PROMETHEE), and geometrical analysis for interactive aid (GAIA). Reference-level modeling involves searching for the alternative that is closest to attaining predefined minimum levels of performance on each criterion (Ruzante, Grieger, Woodward, Lambertini, & Kowalcyk, 2017). The approaches broadly base on linear programming techniques. The choice of the modeling tool can strongly support the design, development and implementation of the decision (Kechagias, Gayialis, Konstantakopoulos, & Papadopoulos, 2020). The most appropriate MCDA model is determined by the objective of the analysis and the nature of decision makers' preferences. Among the most frequently used and implemented methods is the PROMETHEE (Guarini, Battisti, & Chiovitti, 2018). PROMETHEE provides a series of graphic results and comparisons that can be used to discuss findings with the decision makers and assessors (Ruzante, Grieger, Woodward, Lambertini, & Kowalcyk, 2017). Results can be presented as bar graphs, tables or GAIA webs. Decision makers can quickly visualize how the different interventions compare with each other in a specific scenario and/or evaluate the impact of the different scenarios in the final ranking (Ruzante, Grieger, Woodward, Lambertini, & Kowalcyk, 2017).

CHAPTER 3: MATERIALS AND METHODS

3.1 Description of study area

The chemical food safety of maize flour with respect to HM contamination by the hammer milling method in Kampala city was investigated. Maize is an important staple for the urban poor, for those in institutional settings (hospitals, prisons and schools), and internally displaced persons' (IDP) camps (Candia, Saasa, Muzei, & Ocen, 2004). The use of milling machines to locally process foodstuff is all over the place and has become an economically attractive activity both in the urban and rural settings. According to a USAID survey conducted in Uganda, the central region has the highest number of maize millers. Fifty percent of formal maize trade takes place in Kampala, and the World Food Program (WFP) of the United Nations and private traders account for about 20% of domestic maize purchases (Daly, Hamrick, Gereff, & Guinn, 2016). However, data on the potential metal contamination in these products by the milling equipment is lacking.

The study area comprised Kampala, the Capital City of Uganda. The city lies at latitude 0.3476°N and longitude 32.5825°E, and 1223 m (4012 ft) above sea level in the central region of Uganda. It covers a total area of 189 square kilometers (73 sq. miles). Kampala has a tropical climate with an average annual temperature of 21.3°C, and average annual rainfall of *ca.*1,400 mm. The resident population of Kampala is estimated to be 1,936,080 (0.78 male: 1.0 female) inhabitants of whom 16% are children 5 to 9 years of age (Bamuwamye *et al.*, 2017). The city is administered by the Kampala Capital City Authority (KCCA) and is administratively divided into Kampala Central, Kawempe, Makindye, Nakawa & Rubaga divisions (Figure 6).

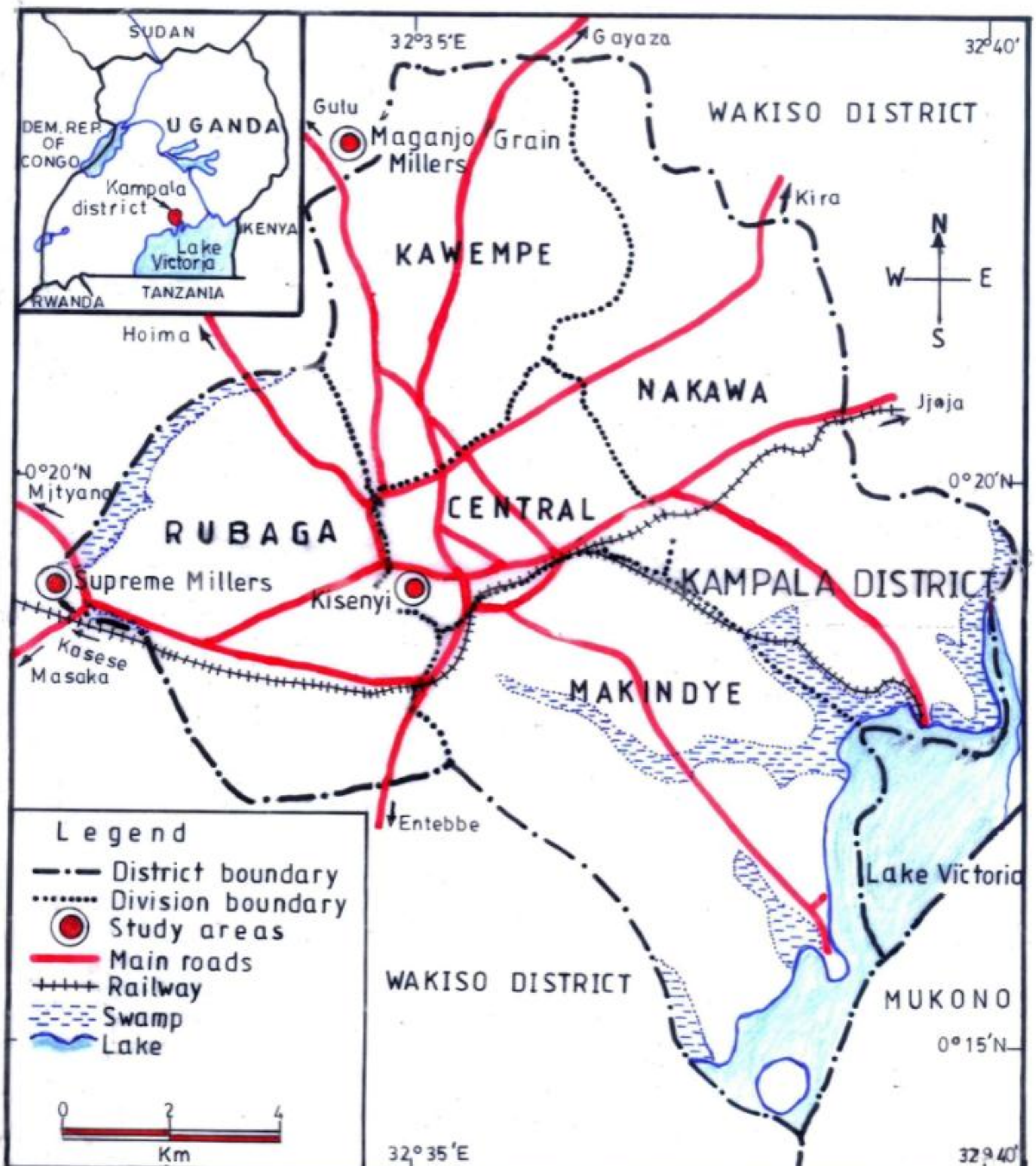

Figure 5: Map of Kampala showing the location of the sampling site

3.2 Methods

3.2.1 The risk-based sampling plan

The risk-based sampling approach described previously by Lahou, Jacxsens, Landeghem, & Uyttendaele (2014) was used to set up the sampling plan. By this approach, the probability that HM appears in the final maize meal due to hammer milling is real and the human health risk due to HM is moderate hence a risk level of 3 × 2 = 4. Maize consumption in Uganda is > 1 kg per person per year, which is considered major and therefore scored 3 points. The risk profile of a country of origin other than the EU, is scored 5 points. The enterprises are not certified for Food Safety Management Systems hence scored 5 points. The volume of production of the millers is less than 25,000 tonnes per year (Shirley, Liu, Kakande, & Kagarura, 2021), which is considered a minor volume and therefore scored 1 point. The total score (risk level) was obtained as 18 points. A risk level between 17 and 22 points is described as high. This information was used to determine the number of samples for analysis (Lahou, Jacxsens, Landeghem, & Uyttendaele, 2014). For a high risk level, the number of samples needed to detect a total positive fraction of 1% at a confidence interval of 95% is 300 per year (Table 4). The number of samples that were analyzed in 2 months is 50. Samples from all sampling sites were evenly divided between the maize grain and maize flour, i.e., 25:25.

Table 4: Risk based sampling plan for maize grain and maize flour

Product	Hazard	A	B	c	d	E	f	G	H
Maize flour	Heavy metal	4	3	5	5	1	18	High	300

a: Risk profile of the product (Risk=Probability × Effect); b: consumption (major/minor); c: Risk profile of region of origin; d: Risk profile of supplier; e: Major or minor volume; f: Total score; g: Risk level; h: Samples per year.

Samples were obtained, and milled at Maganjo Grain Millers, Super Millers in Kawempe, and from Atuha, Ibra and Mbogo maize milling enterprises in Kisenyi (Kampala Central Business District). Sampling was conducted during the months of May and June 2020. Maize grain was dried in a hot air oven at 105°C for four and half hours. The dried grain was ground into fine particles using a porcelain pestle and mortar to avoid metal contamination of the samples. The resulting powder was packed in dry air tight plastic bags and kept under cool dry conditions till analysis. All samples were coded in order to conceal their identity and source of origin.

3.2.2 Sample digestion

Ground maize flour (5.0 g), was weighed out into a clean labelled crucible and dry ashed in a muffle furnace by stepwise increase of the temperature up to 500°C and then left to ash at this temperature for 6 h (Bamuwamye, Ogwok, & Tumuhairwe, 2015). Flour was removed from the furnace and allowed to cool. The ash was wetted with distilled water (1 ml) and concentrated ultrapure HNO_3 (2.5 ml) was added. The crucible was covered with a watch glass and placed on a hot plate. The digestion was performed at a temperature of 95°C for 1 h. The ash was dissolved in 5 ml of 9.25% HCl and digested on hot plate until white fumes ceased to exist. After cooling, 20 ml of distilled water was added and filtration done using Whatman filter No. 41. The filtrate was diluted with distilled water up to the mark in a 50 ml volumetric flask. Samples were prepared in triplicate. The solutions were analyzed for Fe, Cu, Cr and Mn and the concentrations blank corrected. Standard solutions of the metals were also prepared at 5 different concentrations and the absorbance (A) determined. A calibration curve was generated in each case. From the calibration curves, the unknown concentrations of the four analytes in the different samples were determined. Metal concentration in maize and maize flour was recorded in mg/kg wet weight.

3.2.3 Determination of iron, manganese, copper and chromium in maize and maize flour

Iron, Mn, Cu and Cr content was determined according to AOAC First Action Method 2015.01 (AOAC, 2015). Analysis was done on a AA6300-Shimadzu double beam Atomic Absorption Spectrophotometer, AAS (Shimadzu Corporation, Japan) with graphite furnace atomization. The AAS is equipped with a deuterium lamp for background correction and hollow-cathode lamps for each of the studied elements, as well as with an ASC-6100F auto sampler, data acquisition and processing software. The operating conditions of the HM by AAS are shown in Table 5.

Table 5: Instrument operating conditions for determining heavy metal by AAS

Element	Wavelength (nm)	Lamp current (mA)	Slit width (nm)	Flame type
Cu	324.7	5.8	1.00	air/acetylene
Fe	248.3	6.4	0.75	air/acetylene
Mn	279.8	6	0.5	air/acetylene
Cr	357.9	6	0.5	air/acetylene

AAS: Atomic Absorption Spectrophotometry

3.2.4 Exposure and health risk assessment

Risk assessment consisted of exposure assessment, dose-response (toxicity) and risk characterization (Adamu, Nganje, & Edet, 2015). The health risk assessment of each potentially toxic element was done based on the quantification of the risk level and expressed in terms of non-cancer hazard quotient (Sun, Zhang, Ma, & Chen, 2015). The exposure parameters used in the risk assessment process are presented in Table 6.

Table 6: Exposure parameters used for risk assessment through the oral exposure pathway

Parameter	Unit	Child	Adult
Body weight (BW)	Kg	20.5	60.7
Exposure frequency (EF)	days/year	365	365
Exposure duration (ED)	Years	6	70
Ingestion rate (IR)	g/day	200	400
Conversion factor (CF)	kg/g	10^{-3}	10^{-3}
Average time (AT)			
For carcinogens	Days	365×70	365×70
For non-carcinogens	Days	365×ED	365×ED

Source: Bamuwamye, Ogwok, & Tumuhairwe (2015); Kamunda, Mathuthu, & Madhuku (2016).

3.2.4.1 Determination of heavy metal exposure

The average daily maize meal intake or the ingestion rate (IR) was determined using secondary data of the World Health Organization (WHO). The daily intake of HM from the consumption of maize meal was estimated using equation 1 (Copat *et al.*, 2013):

Estimated daily intake of metal (EDI) = C X IR/BWa (1)

where C, IR and BWa represent the metal concentrations in maize meal ($mgkg^{-1}$), ingestion rate for maize meal and average body weight, respectively.

3.2.4.2 Determination of non-cancer risks due to Fe, Mn, Cu and Cr in maize flour

Non-cancer risks due to heavy metal exposures in maize meal were determined using the non-cancer hazard quotient (HQ) described by the US EPA (Copat *et al.*, 2013). Total non-cancer risk expressed as the hazard index was determined as the sum of individual HQ of the different metals. Non-cancer risks are expressed in terms of HQ for a single substance, or hazard index (HI) for multiple substances and/or exposure pathways. Non-cancer risks are assumed to exhibit a threshold below which no adverse effects are expected to be observed. The hazard quotient was calculated using Equation 2.

$$HQ = EDI/RfD \quad (2)$$

Where, EDI is the daily intake rate and RfD is the oral reference dose (mg/kg/day) of the contaminant. The oral reference dose (Table 7), is an estimate of the maximum permissible risk on human population through daily exposure, taking into consideration a sensitive group during a lifetime (Zhang, 2010).

Table 7: Oral reference dose (RfD) in (mg/kg-day) for iron, manganese, copper and chromium

Metal	Fe	Cu	Cr	Mn
Oral reference dose (RfD)	0.7	0.04	1.5	0.14

Source: Copat *et al*. (2013).

Exposure to multiple contaminants results in additive and/or interactive effects. Therefore, to evaluate the potential risk to human health through more than one HM, HI was calculated as the sum of all HQ calculated for individual contaminants for a particular exposure pathway.

3.3 Determination of the best risk mitigation strategy for reducing heavy metal contamination in maize flour due to hammer milling

The best risk management option for controlling metal contamination in maize flour was determined by the Multi-Criteria Decision Analysis (MCDA) using Promethee-Gaia software 1.4 Academic Edition. It involved generating scientific evidence for the best option among different interventions and scenarios that could be used to mitigate the risk (Ruzante *et al*. 2017). The options were assessed according to the following criteria based on a 5 point qualitative ranking:

1. Effectiveness on food Safety/Public Health; this focused on the ability of the risk management option to reduce public health risk. (1=Very low, 2=low, 3=moderate, 4=High, 5=Very high)
2. Food security; ability of the option to maximize food security of the population. (1=Very low, 2=low, 3=moderate, 4=High, 5=Very high)
3. Social acceptability (1=Very bad, 2=Bad, 3=Average, 4=Good, 5=Very good)
4. Infrastructure; institution capacity to implement the proposed option, availability of the managerial structures, technology and storage and transportation facilities, (1=Very bad, 2=Bad, 3=Average, 4=Good, 5=Very good)
5. Associated cost; financial implication of the option, (1=Very low, 2=low, 3=Average, 4=high, 5=Very high)
6. Human resources; the number of technical or trained personnel available, (1=Very bad, 2=Bad, 3=Average, 4=Good, 5=Very good)

The most suitable approach of potential risk mitigation strategies was determined by running the options suggested by the Food and Agricultural Organization (FAO), in Promethee-Gaia software 1.4 Academic Edition. The strategic options are presented in Table 8.

Table 8: Potential risk mitigation strategies for metal contamination in maize flour

Serial number	Intervention	Description
1	Central government programme	Introduce a central government licensing, inspection and training system for maize millers. Only trained and registered millers would be allowed to process maize. This would require strengthening central government institutions and mobilization of the necessary resources.
2	Local government programme	Introduce a local government control through increased training, inspection and enforcement, coupled with a licensing programme. The effect would be similar to central government licensing, but would build on existing local infrastructure and capacities leading to efficiencies.
3	Processor training and certification through a mentorship program	Establish an innovative, community-based processor training and certification system, utilizing available support from regulatory agencies, civil society and manufacturing associations. This relies on training of mentors and the ongoing training and certification commitment from mentors. This will create stakeholder (Local Government Officials, Management of milling enterprises, food relief agencies, machine operators, suppliers of machinery, traders and consumers) understanding and awareness on the importance of food safety and good nutrition.
4	Consumer education	Consumer education leading to informed choices and awareness on the health effects of HM in food. This will create consumer understanding and awareness on the importance of food safety.
5	Combine option 3 and option 4	Combine the community based self-management option 3 and consumer education option 4. Educated consumers are likely to be more motivated to seek certified or recognized maize flour.

Source: FAO (2017)

3.4 Statistical analysis

Data was analyzed in the context of A General Linear model as implemented in SPSS v16 (SPSS for Windows, Version 16.0 (2007), SPSS Inc, Chicago). The concentration of HM for each sample was measured in triplicate. The average concentration was calculated for each HM. The average concentration of each HM was used as the dependent variable in the statistical model. The two-way univariate ANOVA model tested the fixed effects of sample type (Maize seed vs Maize flour), sample source and their interaction on the concentrations of Cu, Cr, Fe and Mn. For the 5 sample sources, adjustment for multiple comparisons was done using the Bonferroni correction.

1

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Heavy metal content of maize flour and maize grain

The concentrations of HM in maize flour and in the control (maize grain), are presented in Table 9. Heavy metal concentration in the grain ranged from 0.257 to 0.988, 0.016 to 0.094, 0.122 to 0.575 and not detected (N.D.) to 0.477 mg/kg for Fe, Cu, Cr and Mn, respectively. In as far as maize flour is concerned, the concentration of the metals was; 0.48 to 1.782, 0.139 to 0.198, 0.159 to 0.501 and 0.059 to 1.151 mg/kg, respectively. Overall, iron was the predominant element in both maize grain and maize flour. Iron concentration was highest in maize grain and maize flour obtained from Atuuha millers. The Fe content of maize flour was significantly ($p < 0.05$) different from the Fe content of the maize grain. There was no pronounced difference in terms of Fe concentration between samples obtained from Atuuha, Ibrah, Super and Supreme Millers. The Fe content of samples from Maganjo and Mbogo Millers showed pronounced difference from the rest of the samples. Chromium concentration was second to Fe in the maize grain, which content increased moderately in maize flour. Manganese was only detected in maize grain samples from Atuuha Millers and Maganjo Millers. However, Mn was detected in all maize flour samples. Maize grain from Ibra had the highest (0.098 mg/kg) content of Cu, while Mbogo had the lowest. On the other hand, maize flour from Super Millers had the lowest concentration of Cu while Mbogo had the highest. There was pronounced ($p < 0.05$) difference between the concentration of Cu in the maize grain and maize flour the same source.

Table 9: Iron, copper, chromium and manganese content (mg/kg) in maize flour and maize grain

Source/sample	Fe		Cu		Cr		Mn	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
MG1	0.987[a]	0.038	0.060[a]	0.002	0.122[a]	0.007	0.365	0.003
MG2	0.651[ab]	0.021	0.091[a]	0.005	0.575[a]	0.019	N.D.	-
MG3	0.67[ab]	0.014	0.089[a]	0.003	0.377[a]	0.009	0.447	0.004
MG4	0.257[b]	0.058	0.016[a]	0.003	0.471[a]	0.005	N.D.	-
MG5	0.875[a]	0.027	0.084[a]	0.003	0.391[a]	0.003	N.D.	-
MF1	1.782[c]	0.058	0.173[a]	0.011	0.443[a]	0.003	0.575	0.018
MF2	1.363[a]	0.034	0.194[a]	0.031	0.399[a]	0.004	1.151	0.028
MF3	0.48[b]	0.015	0.146[a]	0.001	0.359[a]	0.009	0.968	0.033
MF4	1.067[a]	0.019	0.139[a]	0.001	0.159[a]	0.003	0.524	0.013
MF5	1.272[a]	0.025	0.198[a]	0.021	0.501[a]	0.017	0.059	0.001

SD: standard deviation; MG: maize grain; MF: maize flour; N=50, Means with different superscripts in the same column are significantly different ($p<0.05$); n.d. Not detected.

The content of Fe, Cu, Cr, and Mn in maize grain and maize flour was lower than the corresponding permissible limit of 15, 2, 1.3 and 2.3 mg/kg (Larsen, Cobbina, Ofori, & Addo, 2020). Nonetheless, the high concentration of HM in maize flour milled using the hammer mill could be attributed to the wear and tear of the hammer mills as the grains come in contact with it during grinding resulting in its chipping off into the milled sample (Kalagbor, Fyneface, Korfii, Ogaji, & Kpoonanyie, 2017). The high concentration of HM recorded in milled samples in comparison to the control shows a level of contamination introduced by the mill (Oniya *et al.*, 2018).

4.2 Heavy metal exposure in maize flour

In general, HM intake via maize flour is higher when compared with that of the maize grain (Table 10), which could be attributable to an increase in the HM load in maize flour as a result of milling. The highest intake in both maize grain and maize flour was observed for Fe, followed by Cr, Mn and Cu. The EDI for Fe ranged between 0.003 and 0.012 and 0.005 and 0.017 mg/kg/d for adults and children, respectively. The EDI was recorded for Cu as 0.001, and 0.001 to 0.002 mg/kg/d correspondingly for adults and children. Manganese EDI was also observed to be high in milled maize flour both adults and children. The HM intake for children was higher than that for the adults. Children breathe more air, drink more water, and eat more food per kilogram of body weight than adults (Hauptman & Woolf, 2017). This results in greater exposures per kilogram of body weight to any contaminants in the air, water, or food compared with adults. The EDI of the metals were lower than the tolerable upper limits of 40, 10 and 11 mg/d for Fe, Cu and Mn, respectively (Schümann, Borch-Iohnsen, Hentze, & Marx, 2002).

Table 10: Estimated daily intake (mg/kg/d) of iron, copper, chromium and manganese through consumption of maize grain and maize flour for both children and adults

Source/sample	Fe		Cu		Cr		Mn	
	Adult	Children	Adult	Children	Adult	Children	Adult	Children
MG1	0.007	0.010	0.000	0.001	0.001	0.001	0.004	0.006
MG2	0.004	0.006	0.001	0.001	0.004	0.006	0.008	0.011
MG3	0.004	0.007	0.001	0.001	0.002	0.004	0.006	0.009
MG4	0.002	0.003	0.000	0.000	0.003	0.005	0.000	0.000
MG5	0.006	0.009	0.001	0.001	0.003	0.004	0.000	0.000
MF1	0.012	0.017	0.001	0.002	0.003	0.004	0.002	0.004
MF2	0.009	0.013	0.001	0.002	0.003	0.004	0.000	0.000
MF3	0.003	0.005	0.001	0.001	0.002	0.004	0.003	0.004
MF4	0.007	0.010	0.001	0.001	0.001	0.002	0.003	0.005
MF5	0.008	0.012	0.001	0.002	0.003	0.005	0.000	0.001

MG: maize grain; MF: maize flour; 1. Atuuha Millers; 2. Ibrah Millers; 3. Maganjo Millers; 4. Super Millers; 5. Mbogo Millers

4.3 Non-cancer risks of heavy metals in maize flour

The HQ for Fe, Cu, Cr and Mn exposure in maize grain and maize flour are presented in Table 11. The HQ obtained for each metal in this study, was less than 1 for both children and adults. Also, the hazard index (HI) values of the HM for both children and adults were less than 1. According to USEPA (2011), HQ or HI >1 indicate potential adverse health effect, and HQ or HI < 1 would denote a no adverse effect to the health of the consumers. The results of this study, therefore, imply that the population will not experience adverse health effects upon consumption of hammer milled maize flour. These findings differ from those of Adeti (2015) who recorded a HI value of 1.438 for Cr in the maize flour milled using locally fabricated plates in Ghana. Bioaccumulation of toxic elements in the human body resulting from their chronic exposure in milled maize flour may lead to adverse health effects later in life (Larsen, Cobbina, Ofori, & Addo, 2020). Therefore, commercial milling machines should be redesigned with the incorporation of permanent magnets to minimize or totally eliminate the introduction of metal fillings into the ground grains and other foodstuffs.

Table 11: Non-cancer hazard indices for Fe, Cu, Cr and Mn for adults and children

Source/sample	Adults				HI=∑HQ	Children				HI=∑HQ
	Fe	Cu	Cr	Mn		Fe	Cu	Cr	Mn	
MG1	0.009	0.010	0.001	0.027	0.047	0.014	0.015	0.001	0.040	0.069
MG2	0.006	0.015	0.003	0.054	0.078	0.009	0.022	0.004	0.080	0.115
MG3	0.006	0.015	0.002	0.046	0.068	0.009	0.022	0.002	0.067	0.101
MG4	0.002	0.003	0.002	0.000	0.007	0.004	0.004	0.003	0.000	0.011
MG5	0.008	0.014	0.002	0.000	0.024	0.012	0.02	0.003	0.000	0.035
MF1	0.017	0.029	0.002	0.017	0.064	0.025	0.042	0.003	0.025	0.095
MF2	0.013	0.032	0.002	0.000	0.047	0.019	0.047	0.003	0.000	0.069
MF3	0.005	0.024	0.002	0.021	0.051	0.007	0.036	0.002	0.031	0.076
MF4	0.01	0.023	0.001	0.025	0.058	0.015	0.034	0.001	0.037	0.086
MF5	0.012	0.033	0.002	0.003	0.050	0.018	0.048	0.003	0.004	0.073

1. Atuuha Millers; 2. Ibrah Millers; 3. Maganjo Millers; 4. Super Millers; 5. Mbogo Millers; MG: Maize grain; MF: Maize flour

4.4 Multicriteria decision analysis for the selection of an intervention to reduce exposure to HM in hammer milled maize flour

Guaranteeing food safety is an important pillar of food security. It requires consideration of multiple factors that, if taken individually, may seem consequential but which, taken in context, may differ in their relevance to particular decision goals (Garre *et al*., 2020). Five potential interventions were considered for reducing consumer exposure to HM in hammer milled maize flour in Uganda. The interventions were; Central government programmes, Local government programmes, processor training and certification through a mentorship program, consumer education leading to informed choice and a combination of processor training and certification and consumer education. The interventions were ranked against the criteria as shown in Table 12.

Table 12: Comparison of risk management interventions for heavy metals in maize flour

Decision criteria	A	B	C	D	E
Public Health Safety	3	4	4	4	5
Food security	3	4	4	2	4
Social acceptability	2	2	4	4	4
Infrastructure	3	4	4	3	3
Associated cost	4	3	3	2	4
Human resources	4	3	2	4	3

A: Central government programmes; B: Local government programmes; C: processor training and certification through a mentorship program; D: consumer education leading to informed choice; E: combination of processor training and certification and consumer education; 1=Very low; 2=low; 3=moderate; 4=High; 5=Very high.

The PROMETHEE Walking Weights outcome of the intervention is shown in Fig. 7. According to FAO (2017), positive values denote strengths whereas the negative values denote weaknesses. Therefore, consumer education leading to informed food choices and processor training and certification in combination with consumer education emerged as the most suitable risk management options. Central Government programs were likely to play a less significant role, while processor training alone, and the Local Government interventions would probably be ineffective.

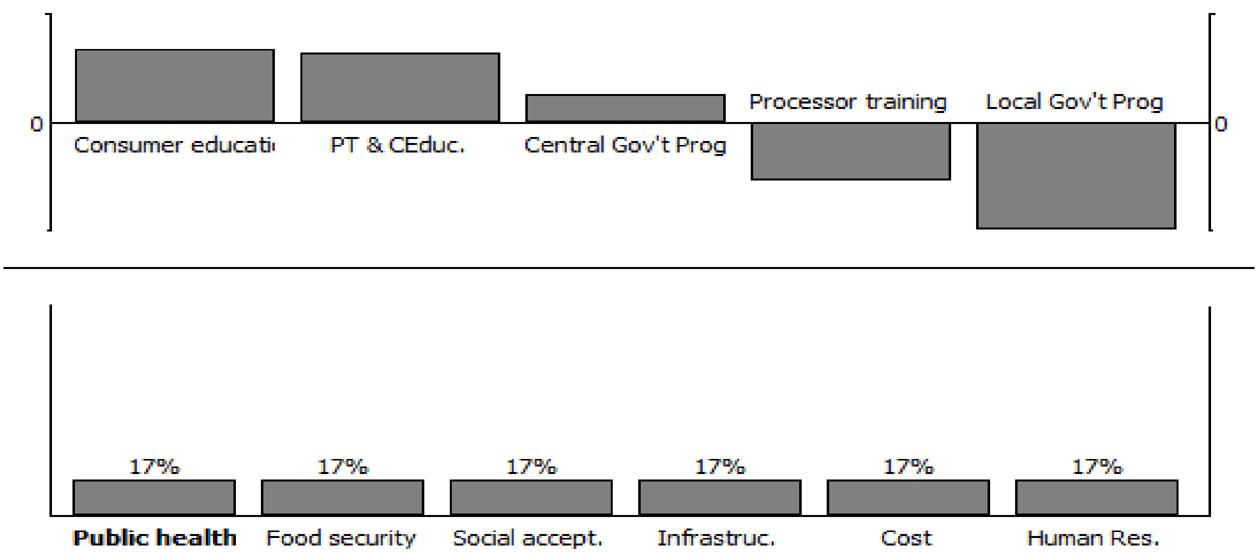

Figure 6: PROMETHEE Walking Weights chart for the interventions: All six criteria were considered equally relevant for decision making in expressing the contributions of decision criteria to the overall strengths and weaknesses of five intervention options for reducing human exposure to HM in maize flour in Kampala.

There are no fully developed strategies for the control of HM contamination in milled food products in Uganda. However, lower levels of heavy metals could be pursued by using good manufacturing and processing practices (Oniya, Olubi, Ayodeji, & Agbi, 2018). For example, materials with better wear resistance should be selected for production of hammers to be used for milling. Formal training of artisans involved in metal works is also necessary to assist in production of quality local mill plates so as to reduce the wearing of plates into milled maize thereby minimizing any health dangers associated with the bioaccumulation of especially Fe in the human body. Therefore, education on sources of food contamination should be organized for stakeholders in the maize cultivation and processing industry, and regulatory systems should be strengthened to safeguard consumers' health (Adu *et al*., 2020).

The MCDA identifies the best option among multiple, potentially conflicting decision alternatives (FAO, 2017). It is a rather new concept in food safety but with a track record in medical, nutritional and environmental decision-making processes. It is recommended by the FAO as an evidence-based, rigorous and transparent process for food safety governance decisions (FAO, 2017) and has been applied to simultaneously evaluate biological and chemical hazards related to emerging dietary practices in France (Eygue *et al*., 2020). Van der Fels-Klerx *et al*. (2018) also emphasized the potential of MCDA for combining different types of information (i.e. quantitative and qualitative) in ranking decision alternatives.

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The health risk and mitigation strategies of Fe, Cu, Cr and Mn in hammer milled maize flour were investigated. Iron and Mn were predominant elements in maize grain, and increased tremendously in the maize flour. The estimated daily intake of the metals was lower than the tolerable upper limit for all the metals. The intake of Fe and Mn through consumption of maize flour was high for both adults and children. The metal hazard quotient and hazard index was lower than the US EPA management level of 1 in all cases. The findings indicate that maize flour milled in hammer mills is safe for human consumption. Consumer education and processor training and certification in combination with consumer education are the best risk management strategies to ensure reduction of health risks due to human exposure to HM in milled maize.

5.2 Recommendations

The bioaccumulation of heavy metals in body organs due to exposure to small amounts of metals in food poses a danger. Therefore, it is recommended that the possible development of risk be monitored on a regular basis with the view of putting in place measures to protect public health. In addition, maize millers should be advised to install metal trapping equipment in order to reduce the heavy metal content in maize flour. Government should facilitate the reduction of tariffs on stainless steel to make it affordable to local manufacturers. Consumers and processors of maize flour be trained and sensitized on the dangers of consuming food contaminated with heavy metals. Further studies are recommended in respect of scaling up the study to cover other products such as millet, cassava and groundnuts.

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