HEALTH RISK ASSESSMENT DUE TO EXPOSURE TO ACRYLAMIDE THROUGH CONSUMPTION OF POTATO CHIPS IN KINONDONI MUNICIPALITY DAR ES SALAAM, TANZANIA

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A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN EPIDEMIOLOGY OF THE SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA

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ABSTRACT

A cross-sectional study was carried out in Kinondoni Municipality from November 2015 to February 2016 to assess health risks associated with exposure to acrylamide through consumption of ready-to-eat potato chips. A total of 600 respondents were randomly selected and interviewed on consumption of ready-to-eat potato chips using semi-structured questionnaire. In addition, 100 ready-to-eat potato chips samples were collected to determine the presence and concentration of acrylamide. Monte Carlo Simulation was used to estimate exposure to acrylamide. Margin of Exposure (MOE) approach was used to characterize the risk based on the No Observed Adverse Effect Level (NOAEL) of 200 µg/kg.bw/day and benchmark dose lower confidence limit for a 10% extra risk of tumours ($BMDL_{10}$) of 180 µg/kg.bw/day for Harderian glands in mice . The levels of acrylamide in ready-to-eat potato chips ranged from 103 µg/kg to 1056 µg/kg with the average value of 326.92 ± 173.43 µg/kg. *Per capita* consumption of ready-to-eat potato chips given as mean and the 95^{th} percentile were 170.98g/day/person and 306 g/person/day, respectively. The estimated dietary intakes of acrylamide for mean and 95^{th} percentile were 0.79µg/kg bw/day and 1.56 µg/kg bw/day, respectively. MOEs calculated using both NOAEL and $BMDL_{10}$ were 253 and 228 for mean exposure and 128 and 115 for 95^{th} percentile exposures, respectively. These MOEs indicates a human health concern on acrylamide for Kinondoni population aged between 15 – 70 years. Chips vendors should be educated on measure to be taken in order to reduce acrylamide formation in ready-to-eat potato chips during processing. Also, efforts should be directed towards conducting a total diet study so as to establish the most accurate data on acrylamide exposure to the consumers.

DECLARATION

I, **Mwingira John,** do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

20/11/2018

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(MSc Candidate)

The above declaration is confirmed;

20/11/2018

Prof. Karimuribo, E.D. **Date**

(Supervisor)

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DEDICATION

This work is dedicated to my beloved wife, Sapiensia Lubuva, my son, Crispin John Mwingira and; my daughter Joan, John Mwingira for their prayers, patience and inspiration during the entire period of my Masters studies.

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LIST OF ABBREVIATIONS AND SYMBOLS

AA	Acrylamide
bw	Body weight
BMDL	Benchmark dose low limit
$BMDL_{10}$	Benchmark dose low limit for a 10% extra risk of tumours
CAC	Codex Alimentarius Commission
°C	Degree Celsius
EFSA	European Food Safety Authority
FAO	Food and Agriculture Organization of the United Nations
FSA	Food Standard Agency
g	Gram
IARC	International Agency for Research on Cancer
ILSI	International Life Science Institute
JECFA	Joint FAO/WHO Expert Committee on Food Additive
kg	Kilogram
LC	Liquid Chromatography
LC/MS	Liquid Chromatography-Mass Spectrometry
LOD	Limit of detection
LOQ	Limit of quantification
MOE	Margin of Exposure
NBS	National Bureau of Statistics
NOAEL	No Observed Adverse Effect Level
pH	Hydrogen ion concentration
P95	95^{th} percentile
SD	Standard Deviation

SNFA	Swedish National Food Agency
SPSS	Statistical Package for Social Sciences
SUA	Sokoine University of Agriculture
TFDA	Tanzania Food and Drugs Authority
µg	Microgram
USEPA	United States Environmental Protection Agency
VEO	Village Executive Officer
WEO	Ward Executive Officer
WHO	World Health Organization of the United Nations

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Acrylamide (CH_2=$CHCONH_2$) is a low molecular weight, highly water soluble organic compound. The compound is commercially produced for production and synthesis of polyacrylamide polymers, which have a wide range of uses as a coagulant in wastewater treatment and clarifying drinking water, soil treatment, grouting agents for the construction of dams, foundations and tunnels, and electrophoresis gel in laboratories as well as a cosmetics additive (Simonne and Archer, 2006; Hogervorst *et al.*;2010). Public health concern about health effects associated with exposure to acrylamide started in 1994 when the International Agency Research on Cancer (IARC) based on laboratory studies, classified acrylamide as ''probable carcinogenic to humans'' (IARC, 1994).

According to paper published by the U.S. Department of Health and Human Services Public Health Service Agency for Toxic Substances and Disease in 2012, human beings may be exposed to acrylamide mainly through tree routes: oral, dermal and inhalation and that oral is the primary route of exposure for general population through ingestion of contaminated food. The concern about acrylamide exposure increased in 2002 when it was discovered by the Swedish scientist that acrylamide is present in commonly consumed carbohydrate-rich foods, such as French fries and potato chips (SNFA, 2002). The JECFA, stated that the margin of exposure for dietary acrylamide evaluated based on dietary exposure for eight countries indicated human health concern and that potato chips was the major food contribution to total mean dietary acrylamide exposure (European Commission, 2017; EFSA Panel on Contaminants in the Food Chain, 2015).

Report published by Potato Pro. Com in 2008, demonstrated that in East Africa, consumption of potato chips has been increasing tremendously in urban areas like Kinondoni Municipality due to urbanisation, the proliferation of fast-food restaurants, growing tourism, and a significant change in eating habits among both high- and low-income groups (Potato Pro. Com, 2008).

1.2 Research problem and justification

According to the report from the Joint FAO/WHO Consultation on Health Implications of Acrylamide published in 2011, dietary acrylamide exposure assessment evaluated at international level did not represent African (FAO/WHO, 2011). From the same report it was pointed out that Africa was not included in the evaluation due to the fact that in African countries like Tanzania, the is limited information on concentration of acrylamide in food and human exposure status. This study therefore, was carried out to estimate the acrylamide intake through consumption of ready-to-eat potato chips in Kinondoni Municipality and assess the possible health risks due estimated exposure.

The findings of this study may be useful to food regulatory bodies, universities and research institutions. Food regulators such as the Tanzania Food and Drugs Authority (TFDA) and the Tanzania Bureau of Standard (TBS) may decide to establishing monitoring and surveillance programme on the concentration of acrylamide in the wide range of food products and institute control measures to minimize intake of excessive amount of acrylamide in Kinondoni Municipality and Tanzania at large. Academic and research institutions may undertake a total diet study to provide reliable estimates on dietary acrylamide and their health risks to general population exposure accurate date.

1.2 Objectives

1.2.1 Main objectives

The main objective of this study was to evaluate dietary exposure to acrylamide through consumption of ready-to-eat potato chips in Kinondoni Municipality and assess health risks associated with this exposure.

1.2.2 Specific objectives

The specific objectives of the study were as follows:-

i) To determine levels of acrylamide in ready-to-eat potato chips at food establishments.

ii) To estimate health risks associated with acrylamide exposure via consumption of ready-to-eat potato chips.

1.2.3 Research questions

a) Research question for specific objective (i)

i) What is the concentration of acrylamide in ready-to-eat potato chips at food establishments?

ii) Is there any difference in acrylamide levels in potato chips based on their varieties?

iii) Is there any difference in acrylamide levels in potato chips between sources of samples?

b) Research questions for specific objective (ii)

i) What is *per capita* consumption of ready-to-eat potato chips in Kinondoni Municipality?

ii) Is there any difference in *per capita* consumption of potato chips between males and females?

iii) Is there any difference in *per capita* consumption of potato chips between age groups?

iv) Is there any difference in *per capita* consumption of potato chips among individuals in Kinondoni based on their occupation levels?

v) To what extent do individuals with the age range of 15 to 70 years get exposed to acrylamide via consumption of ready-to-eat potato chips?

vi) Does exposure to acrylamide due to consumption of ready-to-eat potato chips indicate a public health concern?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Sources of acrylamide exposure

Acrylamide has been reported to be found in variety of sources. Certain foods such as potato and grain products or coffee have been reported to be potential sources through which human beings can be exposed to acrylamide through ingestion (SNFA, 2002). Tobacco has also been identified as another source of acrylamide and that it could probably be the major way by which cigarette smokers are exposed to acrylamide (Bergmark, 1997). Apart from food and tobacco, a report published by CODEX in 2004 shows that people who work in certain industries (particularly in the paper and pulp, construction, foundry, oil drilling, textiles, cosmetics, food processing, plastics, mining, and agricultural industries) may be exposed to acrylamide in the workplace, mainly through skin contact or by breathing (FAO/WHO, 2004). Furthermore, it has been reported that exposure to low levels of acrylamide might result from the presence residual acrylamide in polyacrylamide used in cosmetics, water treatment and soil conditioners (Schettgent *et al.,* 2003).

2.2 Acrylamide formation in food

For the first time, acrylamide was reported to be contained in some heat-treated starch-rich foods such as potato and cereal products and coffee by the Swedish researchers (Tareke *et al*., 2002; Svensson *et al*., 2003; Surdyk *et al*., 2004). The results was then confirmed by numerous in different countries. (Ahn *et al*., 2002; Stadler *et al*., 2002; USFDA, 2002; Zhang *et al*., 2005; Mizukami *et al*.,2006; Medeiros *et al*., 2012; Friedman, 2015). It is well now established that acrylamide formation is common in carbohydrate-rich foods cooked (baked or fried) at high temperature usually above 120°C and low moisture and that that free amino acids, mainly asparagine, and reducing sugars are important precursors to acrylamide formation in foods (Svensson *et al.* 2003; Surdyk *et al.* 2004; Keramat *et al.,* 2011;Medeiros *et al.*, 2012; Pedreschi *et al.*, 2014). These researchers argued that under these conditions, the free amino acid asparangine reacts with reducing sugar or other carbonyl compounds to form acrylamide. Even though it has been shown that a temperature of 120 °C or higher is needed for the formation of acrylamide, there are reports confirming that this compound can be formed at temperatures below 100 °C, particularly in drying processes at 65–130 °C (Eriksson, 2005; Biedermann *et al*, 2003).

One potential mechanism that has been linked with acrylamide formation is referred to as the Milliard reaction (Tareke et al, 2000; Stradler et al, 2002; Biederman et al, 2003; Couplin JR, 2003; Yaylayan and Stadler,f 2005). Apart from the Milliard reaction, it has been reported by FAO/WHO (2011) that there are other mechanisms of acrylamide formation such as formation through pyrolysis of wheat gluten or via enzymatic decarboxylation of asparagine in raw potatoes. However, the same source declared that these other routes are of minor importance as far as formation of acrylamide in food is concerned.

2.3 Acrylamide levels in food

Since the first report on the occurrence of acrylamide in food, a range of levels have been reported to be detected in different heat-treated foods in different countries. Regardless of the differences in acrylamide levels, most of the publications reported that fried potato products such as potato crisps, French fries and ready-to-eat potato chips contain higher levels of acrylamide as compared to other food categories (FAO/WHO, 2002; Borda and Alexe, 2011; FSA, 2012). For instance, Becalski *et al.* (2004) and EFSA (2015) reported that ready-to-eat potato chips and potato crisps contain up to 3000 µg/kg or even 4000 μg/kg of this contaminant. Report from JECFA also shows that the range of mean acrylamide levels in potato crisps, French fries, biscuits, crisp bread and crackers and coffee in many countries were 399-1202 μg/kg, 159-963 μg/kg, 169 – 518 μg/kg, 78-459 μg/kg and 3-68 μg/kg, respectively (FAO/WHO, 2011).

2.4 Factors influencing Acrylamide in foods

The most important factors reported include amount of reducing sugars, processing conditions, pH, moisture content and the use of additives (Keramat *et al.*, 2011b; Krishnakumar and Visvanathan., 2014; Liu *et al.*, 2015; Przygodzka *et al.*, 2015).

2.4.1 Effect of reducing sugars

Reducing sugars (glucose and fructose) and free asparagines are the major determiants of acrylamide formation in potatoes (Haase et al., 2003; 2003; Becalski et al.. 2004) . Reducing sugars concentration in potatoes has been found to exhibit a strong correlation with the amount of acrylamide formed in processed potato products (Amrein *et al.*, 2003; Becalski *et al.*, 2004, Amrein *et al.*, 2004, Matthaus, *et al.*, 2004).

2.4.2 Effect of processing conditions

The important processing conditions that influence the process of acrylamide formation are: heating temperature and time, blanching and frying (Ciesarová *et al.*, 2006).

Pedreschi *et al* (2004), investigated the effect of time and temperature on acrylamide formation in fried potatoes and conclude that at a temperature range between 150 °C to 190 °C, frying time and temperature directly affect acrylamide formation. However, other researchers have demonstrated that at higher temperatures (>200 °C), increase in temperature combined with prolonged time reduce the levels of acrylamide since acrylamide formation is also dependent on initial sugar and asparagine contents (Biedermann and Grob , 2003;Fadwal et al, 2009).

Studies conducted to investigates the effect of blanching on the concentration of acrylamide in potato products have concluded that blanching process caused significant decreases of acrylamide levels in processed potato products. For instance, Dorin et al (2014) and Haase et al. (2003), investigated the effect of blanching process on acrylamide formation in potato crisps and concluded that that blanching process caused an average of 60% reduction in acrylamide content in the crisp.

Intensive frying has shown to be correlated to brown colour development as well as acrylamide formation in potato products which occurs in Maillard reaction during heating process, mostly at the end of the frying process (Fishelier *et al.*, 2006; Amrein *et al.*, 2007). According to research conducted by Granda et al (2004), frying under low pressure conditions using vacuum fryer has shown to cause higher reduction in acrylamide formation in fried potato products

2.4.3 Effects of additives

The impact on acrylamide formation of several additives have been investigated in several occasions. For example, investigations on the influence of asparaginase (an enzyme that converts asparagine into ammonia and aspartic acid) on acrylamide formation in potato products have that this enzyme reduces the amount of acrylamide formation in fried potato products (Kumar *et al.*, 2004; Pedreschi *et al.*, 2008; Hendriksen *et al.*, 2009; Medeiros *et al.*, 2010; Pedreschi et al., 2010). Furthermore, addition of other additives such as cysteine and methionine, amino acids or protein rich substances and sodium chloride have shown to reduce significantly the amount of acrylamide formed in fried potato products decreased acrylamide (Rydberg *et al.*, 2003, Levine and Smith, 2005; Voelker L, 2005).

2.5 Exposure and risk to humans

Human exposure to acrylamide may occur through inhalation via smoking tobacco, skin contact or consumption of contaminated food or water (FAO/WHO, 2004; Simonne and Archer, 2006). However, existing data show that dietary exposure accounts for a significant contribution to total acrylamide exposure (FAO/WHO, 2002). According to recent information published by JECFA, it is estimated that the average and high international acrylamide exposure are 1 μg/kg bw/day and 4 μg/kg bw/day, respectively and that, the mean dietary exposure range to acrylamide estimated to be 0.2-1.0 μg/kg bw/day for the general adult population while 95th percentile range is 0.6-1.8 μg/kg bw/day (FAO/WHO, 2011). Fried potato products have been reported to account for significant contribution to dietary acrylamide exposure in several publications (European Commission, 2017; EFSA Panel on Contaminants in the Food Chain, 2015).

2.6 Toxicity

Experimental studies conducted in animals revealed that acrylamide has shown to induce tumours in animals and thus, the International Agency for Research into Cancer (IARC) has classified it as "probably carcinogenic for humans" (IARC, 1994). This classification was internationally authorized by JECFA in June 2002 (FAO/WHO, 2002). In addition, acrylamide has been reported to induce genetic mutations and chromosomal abnormalities in experimental animals (Capuano and Fogliano, 2011). Furthermore, acrylamide has been reported to be associated with increase in incidence of tumour of mammary glands, oral cavity and reproductive tracts in experimental animals (Friedman *et al.*, 1995; Wang *et al.*, 2010). JECFA evaluated the safety of acrylamide for the first time in 2005 and re-evaluated it in 2010 and reported that, it is well-established that acrylamide is neurotoxic in humans as revealed by the consequences of occupational and accidental exposures (FAO/WHO, 2011). However, in the same report JEFCA has cautioned that no proof has been provided by epidemiological studies that occupational exposure or dietary exposure to acrylamide is associated with cancer in humans.

2.7 Exposure assessment approach

The guidance on how to conduct risk assessment of chemicals in food is provided by the International Programme on Chemical Safety (IPCS) monograph on Principles and Methods for the risk Assessment of Chemicals in Food (IPCS, 2009a). The guidelines describe risk assessment as a scientifically based process that consists four steps: hazard identification, dose response assessment, exposure assessment and risk characterization. The guidelines also provide guidance on conducting and interpreting dietary exposure assessment of chemicals in food. Among other things, the guidelines require exposure assessment to cover the general population and as well as critical groups that are significantly different from those of the general population. In principle, dietary exposure combines food consumption data with data on concentration of chemicals in food. The resulting dietary exposure may then be compared with the relevant health guidance as part of risk characterization. Data on food consumption and chemical contamination are usually combined using either a deterministic approach, also called "point estimate", or a probabilistic approach (Kroes *et al.*, 2002). The "point estimate approach" relies on the fixed values on consumption and concentration of the chemical in that food. The major limitation of this method is that it does not reflect the exposure of the overall population. Contrary to a "point estimate approach", probabilistic approaches uses the full distribution of chemical occurrence and consumption data, thus exploiting the variability and uncertainties in both quantities (IPCS, 2009b). Variability in dietary exposure is often described using frequency plots or sometimes frequency distributions.

Regarding risk characterization, it is recommended that for substances that are both genotoxic and carcinogenic like acrylamide (that have no sufficient data to establish a health based guidance value), the risks associated with those substances should be characterized using the margin of exposure (MOE) approach (FAO/WHO, 2002). This approach provides advice to inform risk managers of how close human exposures are to those anticipated to produce a measurable effect in laboratory animals or humans (EFSA, 2005; FAO/WHO, 2005; O'Brien *et al.*, 2006). MOE approach has been supported and used by several advisory bodies such as JECFA, European Food Safety Authority (EFSA) and International Life Science Institute (ILSI) Europe Expert Group (O'Brien *et al.*, 2006; EFSA, 2005).

2.8 Safety reference values and risk characterization approach

By considering that to date there are no acceptable limits for dietary acrylamide consumption that have been established internationally (Simonne and Archer, 2006; Krishnakumar and Visvanathan, 2014), JECFA recommended that it is appropriate to use the MOE approach in assessing the risk of acrylamide exposure to humans. (FAO/WHO, 2002, 2011). With this approach, the risk is estimated based on the benchmark dose lower (BMDL) confidence limit associated with a 10 % extra risk of tumours ($BMDL_{10}$) in rates or No Observed Adverse Effects Level (NOAEL) based on morphological changes in nerves in rats. For genotoxic carcinogens like acrylamide, a MOE value exceeding 10,000, based on $BMDL_{10}$ from the animal study, would be of low concern from public point of view and vice versa.

2.9 Previous exposure assessment studies

The safety of acrylamide has been evaluated several times by food safety scientific bodies and other scientists. For instance, JECFA evaluated the safety of acrylamide for the first time in 2005 and re-evaluated in 2010 (FAO/WHO, 2011). In all cases, dietary intake were estimated at 1 and 4 µg/kg bw/day for general population and consumers with high intake respectively. From the same report, MOE values estimated based on NOAEL of 200 µg/kg bw/day for morphological changes in nerves, and $BMDL_{10}$of 180 µg/kg bw/day for Harderian gland tumours in male mice were 200 and 50, respectively. Risk assessment conducted in Canada in the year 2012 using the same NOEL and $BMDL_{10}$ values used by JECFA, reported to estimate MOE values higher than those estimated by JECFA (Health Canada, 2012). Regardless of differences in MOE values estimated by JECFA and Health Canada, both bodies were of the opinion that dietary exposure to acrylamide represents a potential human health concern.

From the reviewed literature, it is clear that whereas health risk assessment of acrylamide due to consumption of ready-to-eat potato chips have been conducted elsewhere, there is no similar study that has been conducted in Tanzania. Moreover, it is clear that exposure to this contaminant reported elsewhere may not necessary be the same in Tanzania. With respect to exposure assessment and risk characterization approaches, it is clear that probabilistic approach was appropriate for dietary exposure assessment whereas MOE was appropriate for risk characterization respectively.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

The study was conducted in seven wards of Kinondoni Municipality in the Dar es Salaam City, Tanzania. According to Dar es Salaam city profile, the Municipality is bordered by the Indian Ocean to the North east, Ilala Municipality to the South, Bagamoyo district to the North, Kibaha district to the West and Kisarawe district to the South west. The total area of the Municipality is 531 sq. km with its centroid coordinates being 6°45'00" South and 39°10'00". The Municipality experiences a modified type of tropical climate. It is generally hot and humid throughout the year. The hottest season is from October to April while it is relatively cool between May and September. Rainfall in the district is bimodal; the short rains fall between October and December while long rains fall between March and May. According to the Population and Housing Census conducted by the National Bureau of Statistics in 2012, the human population was 1 775 049 people among them 860 802 were males and 914 247 were females (NBS, 2013).

The distribution and location of the study wards namely; Magomeni, Ndugumbi, Makurumla, Mbezi Juu, Goba, Tandale and Ubungo is shown in Figure 1. Considering the population distribution in the study wards, the NBS report shows that Makurumla had the highest number of people (63 355), followed by Ubungo (56 015), Tandale (54 781), Goba (42 669), Mbezi Juu (41 340), Ndugumbi (36 841) and Magomeni (24 400).

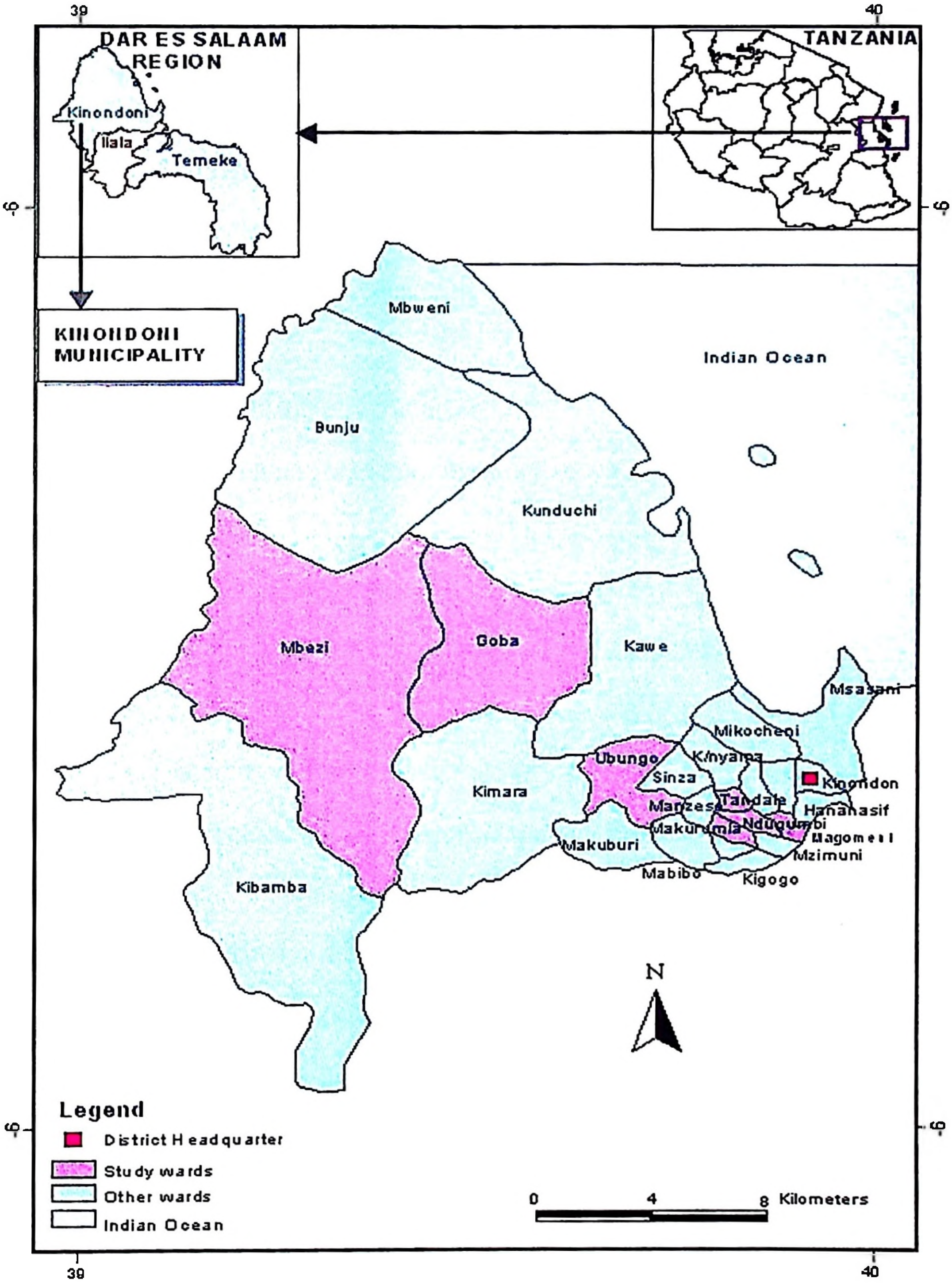


Figure 1: Kinondoni Municipality map showing the study wards

3.2 Study design

A cross-sectional study was conducted from December 2015 to February 2016. The study focused on estimating health risk(s) associated with exposure to acrylamide through consumption of ready-to-eat potato chips. Methods for data collection were based on face to face administration of a structured food frequency questionnaires at individual respondent level and sample collection form food establishment level.

3.3 Sample size estimation and allocation

3.3.1 Ready-to-eat potato chips consumption data

The sample size for respondents involved in the interview regarding ready-to-eat potato chips consumption was estimated using the formula; $n = 1/(1+N\,(e)^2)$ based on Yamenes (1967) as cited by Mhauka *et al.* (2014) where, N represents population size and e represents level of precision. Using the population size (N) of 1 775 049 (NBS, 2013) and the level of precision (e) of 0.05, this resulted in a sample size of 400 individuals. Since cluster sampling technique was used, estimated sample size was multiplied by the design effect of 1.5 in order to achieve the same precision. For this matter then, a total sample size of 600 was considered suitable for this study.

The sample size was further computed using the method of proportionality allocation under which the size of the representative samples from different wards were kept proportion of the ward (Kothari, 2004) using the formula n.Pi. where, Pi = Ni /Nt (Ni represents cluster population and Nt represent total population in Kinondoni municipality). That was if Pi represented the proportion of the population included in the cluster i, and n represented the total sample size. The total sample size from the seven awards is presented in Table 1 below.

Table 1: Distribution of sample size by study wards

Ward	Population (N)	Proportion (P)	Sample size (n)
Goba	42,669	0.13	80
Magomeni	24,400	0.08	46
Tandale	54,781	0.17	103
Ndugumbi	36,841	0.12	69
Ubungo	56,015	0.18	105
Mbezi Juu	41,340	0.13	78
Makurumla	63,352	0.20	119
TOTAL	**319,398**	**1.00**	**600**

3.3.2 Ready-to-eat potato chips sample

The number of samples of ready-to-eat potato chips was estimated by the formula $n = (z^2\sigma^2)/e^2$ (Kothari, 2004), using the value of standard variate (z) of 1.96, standard deviation (σ) of 10 and level of precision (e) of 2, which resulted in the sample size of 97 that was approximated to 100 samples.

3.4 Sampling procedures and techniques

A multi-stage cluster sampling approach was employed to select the respondents. According to Kothari (2004), seven wards (Goba, Magomeni, Makurumla, Mbezi Juu, Ndugumbi, Tandale and Ubungo) located in Kinondoni Municipality were considered as different clusters. A total of 600 households were visited and one respondent per household was included in the study. Systematic random sampling method was used to select respondents. The following procedure was applied to get the respondents in each ward. First the, the central point; a place where approximately an equal number of households were found was identified. Then, by spinning a pencil on a clipboard, a direction was identified and the first 15 households from the identified direction were identified. The first household for sampling was selected by simple random sampling method out of 15 identified households and after that, every fifteenth household was identified for inclusion into the study on an approximately straight line pattern in each ward. Where there were no respondents in the identified households a substitute was taken from the nearest household. If there were two or more eligible respondents in a household, one of them was selected by lottery method. This procedure was continued until the required sample was achieved.

Ready-to-eat potato chips samples were purchased from randomly selected food establishment located in the selected areas based on the list of the establishment obtained from Street Executive Officer.

3.5 Data collection procedures

3.5.1 Recruitment and training

Two research assistants were recruited and trained for two days. The training included objectives of the study, sampling method, data collection procedure, importance of confidentiality and necessary ethical procedures. The assistants also participated in pre-testing of questionnaire and food sampling forms.

3.5.2 Pre-testing of sample collection tools

Before the study, questionnaire and sample collection forms were pre-tested at Mabibo ward in Kinondoni Municipality to check whether the questionnaire and sample collection forms were understood and if the sequence of questions were logical. After pre-testing the questionnaire and sampling forms, the tools were modified accordingly.

3.5.3 Collection of ready-to-eat potato chips consumption data

A total of 600 respondents were interviewed using semi-structured food frequency questionnaire in seven wards (Magomeni, Goba, Tandale, Ndugumbi, Ubungo, Mbezi Juu and Makurumla) of Kinondoni Municipality from November, 2015 to February, 2016. The survey involved face-to-face questionnaire administration at convenient place agreed by the respondents. The questionnaire (Appendix 1) was designed to investigate the ready-to-eat potato chips consumption pattern at individual level. The questionnaire was translated into Swahili, which is the national language for Tanzania (Appendix 2). The general aspects assessed included the intake of ready-to-eat potato chips, variety of ready-to-eat potato chips consumed, amount taken per week and portion size per serving. Demographic variables recorded were respondents' age, sex, weight, education level and occupation. Weight of respondents was taken using mechanical personal scale DT01mechanic Model KINLee manufactured by Zhongshan Jinli Electornic Weighing Equipment Co. Ltd, Zhonghshan Guangdong, China.

3.5.4 Collection of ready-to-eat potato chips samples

A total of 100 ready-to-eat potato chips samples (50 ready-to-eat plain potato chips and 50 ready-to-eat potato chips mixed with eggs) were purchased from the randomly selected food establishments (chips kiosks, restaurants and hotels) located in the study area from November 2015 to February 2016. Distribution of the food establishments from which samples were purchased is summarised in Table 2. Sampling of chips was distributed over the study period The samples were placed into sealed food grade plastic bag labelled with the sample identification number, sampling point and date of sampling. The same information was also recorded in the food collection form (Appendix 3). Each portion of ready-to-eat potato chips was measured using digital balance Fisher Portable balance model SG-2001 (Fisher Scientific Ltd, Ottawa) to obtain the actual weight (g). The samples were then stored at – 20 °C before analysis.

Table 2: Distribution of ready-to-eat potato chips samples by varieties and location

Ward	Number of samples (n)		
	Ready-to-eat plain chips	Ready-to- eat potato chips mixed with eggs	Total
Goba	4	4	8
Magomeni	9	9	18
Tandale	9	9	18
Ndugumbi	6	6	12
Ubungo	9	9	18
Mbezi Juu	4	4	8
Makurumla	9	9	18
TOTAL	50	50	100

3.6 Laboratory analysis

3.6.1 Chemical analysis of acrylamide

Laboratory analysis of acrylamide was conducted at the Tanzania Food and Drugs Authority (TFDA) laboratory in Dar es Salaam, Tanzania using Liquid Chromatography Mass Spectrometry (LC/MS) method. The analytical method used was described by Takatsuki *et al* (2003). In brief, acrylamide was extracted with a mixture of water and acetone from homogenised potato chips samples. The extracts were concentrated, washed with dichloromethane for defatting and cleaned up on BondElut and Accucat cartridges. The final acrylamide extracts obtained were injected in LC/MS and analyzed in the selected ion recording (SIR) mode. For the LC/MS analysis, four LC columns were connected in series and the flow of the mobile phase was switched according to a time-programme. The limit of detection (LOD) and limit of quantification (LOQ) for acrylamide in the samples were 0.009µg/g and 0.003µg/g, respectively.

3.6.2 Analytical quality assurance

The analytical method was validated by single-laboratory validation against certified reference materials purchased from Sigma-Adrich Chemical Company Germany. Average recoveries ranged between 99.5 % and 100 %.

3.7 Determination of acrylamide levels in ready-to-eat potato chips

A total of 100 ready-to-eat potato chips (50 plain ready-to-eat potato chips and 50 chips mixed with egg were analyzed for determination of acrylamide content. Descriptive statistics (means, standard deviations, medians and ranges) were computed using Statistical Package for Social Sciences version 23 (IBM SPSS Corporation, Chicago). The mean and 95th percentile average concentration from each variety of ready-to-eat potato chips were used to present average and high concentration of acrylamide in ready-to- eat potato chips.

3.7.1 Establishment of daily intake of ready-to-eat potato chips

Information on ready-to-eat potato chips consumption was collected by trained research assistants using the food frequency questionnaire. The questionnaire included questions about frequency of consumption of ready-to-eat potato chips per week. Consumption frequency for each variety of ready-to-eat potato chips was measured as "once per week", "twice per week", "after every other day" and "every day". During analysis, data on consumption frequency were transformed to serving per week. It was assumed that times could be equated to portion. Therefore, the frequency of consumption was transformed as follows: "once per week" was transformed to "1 time per week", "twice per week" transformed to "2 times per week", "after every other day" became "3.5 times per week" and "every day" became "7 times per week". *Per capita* consumption of ready-to-eat potato chips of an individual was computed by combining average weight of ready-to-eat potato chips portion and frequency of consumption as indicated in equation (1)

$$IR = P \times F/7days \qquad (1)$$

Where IR represents iindividual daily consumption of ready-to-eat potato chips (g/day), P denotes average weight of a plate (portion) of ready-to-eat potato chips (g) and F represents frequency of consumption of ready-to-eat potato chips in a week for an individual.

3.7.2 Inclusion and exclusion criteria

Inclusion and exclusion criteria for the ready-to-eat potato chips consumption survey included:

c) Inclusion Criteria

- Age between 15 years and 70 years
- Study area residents
- Respondent willingness to participate

d) Exclusion Criteria

- Age below 15 years or above 70 years
- Non-residents of the study areas
- Respondent unwillingness to participate.

3.7.3 Risk assessment approach

Risk assessment was conducted based on the definition and methodology of the International Programme on Chemical Safety framework (IPCS, 2009a). The methodology describes risk assessment as a scientifically-based process consisting of four steps; hazard identification, hazard characterization, exposure assessment and risk characterization. However, dose response assessment was not carried out in this study because the study focused on one route only (dietary exposure). Dose response data established by JECFA in 2010 was used to characterize the risk.

3.7.4 Exposure assessment

(a)Exposure model

The daily intake (DI) of acrylamide from consumption of ready-to-eat potato chips was estimated by combining data on acrylamide in ready-to-eat potato chips, potato chips consumption rate and body weight of respondents in equation (2):

$$DI = IR \times C_{AA} / BW \qquad (2)$$

Where DI denotes the daily intake of acrylamide from consumption of ready-to-eat potato chips (μg/kg bw/day); IR represents estimated ingestion rate of ready-to-eat potato chips (g/day); C_{AA} represents the concentration of acrylamide (μg/kg).

(b) Probabilistic estimation of dietary exposure to acrylamide

The probabilistic assessment of the exposure to acrylamide due to consumption of ready-to-eat potato chips was achieved by performing Monte Carlo Simulation on the basis of guiding principles for Monte Carlo analysis (EPA, 1997). Both input and output variables were fitted in the suitable distribution in the dietary exposure equation (2). Normal distribution was the best fitting distribution to this equation. The average and 95th percentiles of the exposure levels were used to present the average dietary exposure and the exposure for the high consumers, respectively. The @ Risk software, version 7 Palisade Corporations, New York City was used to fit in the exposure model inputs to probability function with 10,000 iterations. Fitted distributions for the input variables are presented in Table 3.

Table 3: Fitted distribution for the input variables (acrylamide concentration, ready-to-eat potato chips consumption data and body weight)

Variable		Distribution
Acrylamide content in potato chip (μg/kg)	Plain chips	Normal (282.78, 169.17)
	Chips mixed with egg	Normal (371.06, 167.86)
	Both plain chips and chips mixed with eggs	Normal (326.92, 173.34)
Body weight(kg)	Youths	Normal (66.18, 7.91)
	Adults	Normal (76.90, 7.15)
	Elderly	Normal (89.30, 9.578)
Consumption of plain ready-to-eat potato chips (g/person/day)	Youth	Normal (97.12, 56.81)
	Adult	Normal (81.12, 53.72)
	Elderly	Normal (57.41, 36.06)
Consumption of chips-mayai (g/person/day)	Youth	Normal (102.47, 56.97)
	Adults	Normal (93.11, 57.71)
	Elderly	Normal (45.10, 29.01)
Consumption of both (plain ready-to-eat potato chips and chips mixed with eggs (g/person/day)	Youth	Normal (199.59, 79.27)
	Adult	Normal (174.23, 72.93)
	Elderly	Normal (102.51, 44.42)

3.7.5 Risk characterization

Based on the fact that acrylamide is classified as a potential carcinogenic compound, risk characterization was conducted according to the harmonized approach of the JECFA (FAO/WHO, 2011), which recommends the use of margin of exposure (MOE) using (1) NOAEL of 200 μg/kg bw /day based on morphological changes in rats; (2) a $BMDL_{10}$ of 180 μg/kg bw /day for Harderian gland tumours in male mice.

MOE values were calculated by comparing the NOAEL and $BMDL_{10}$ value to the dietary exposure estimates for acrylamide according to the following (Equation 3):

$$MOE = NOAEL \text{ or } BMDL_{10}/\text{Acrylamide Exposure estimates} \quad \ldots\ldots\ldots\ldots\ldots (3)$$

Small MOE values (< 10 000) were considered to indicate high public health concern whereas large MOE values (≥10 000) were considered to indicate low public health concern (EFSA, 2007:, Benford *et al.*, 2010).

3.8 Statistical analysis

Data was first entered and cleaned in Microsoft Excel 2007 (Microsoft Corporation, Washington) and imported into Statistical Package for Social Sciences version 23 (IBM SPSS corporation, Chicago) for analysis. The study employed a three step analysis. In the first step, descriptive statistics were computed to determine ready-to-eat potato chips consumption data and concentration of acrylamide in ready-to-eat potato chips. Descriptive statistics are presented as means (95% Confidence Interval), standard deviations, medians, minimum, maximum and 95^{th} percentiles. One-way Analysis of Variance (ANOVA) was performed at a critical p value of <0.05 to determine if there were statistical differences in the mean potato chips consumption between gender, age groups and occupation status. The same test was used to determine if acrylamide levels in potato chips were different based on their mode of preparation. In the second step, Monte Carlo simulation was employed using @ Risk software version 7 (Palisade Corporation, New York) to estimate dietary intake of acrylamide via consumption of ready-to-eat potato chips. The third stage involved computation of the MOE based on based on the No Observed Adverse Effect Level (NOAEL) of 200 µg/kg.bw/day and benchmark dose lower confidence limit for a 10% extra risk of tumours ($BMDL_{10}$) of 180 µg/kg.bw/day for Harderian glands in mice for assessing the risk of acrylamide exposure.

3.9 Ethical considerations

A research clearance letter was obtained from the Vice Chancellor of the Sokoine University of Agriculture (Appendix 4). Permission to carry out the study in the Municipality was granted by the Kinondoni Municipal Director (Appendix 5). Participation in the study was based on voluntary basis. Before questionnaire administration, the interviewer explained the purpose and objective of the study, importance and possible outcomes and, thereafter asked for permission to administer the questionnaire. High confidentiality and anonymity was observed during filling the questionnaires. The respondent name was not included in the questionnaire but a code known only to the interviewer for identification if needed was used. All respondents involved in the study were asked for verbal consent to administer the questionnaire and were assured from the risk of damage from results of the study.

CHAPTER FOUR

4.0 RESULTS

4.1 Demographic characteristics of the respondents

Demographic characteristics of the respondents are presented in Table 4. The study involved 600 residents from Kinondoni Municipality. The findings show that proportions of male and female respondents were almost the same. The results in Table 4 also show that the proportion of elderly people was the lowest compared to other categories and it includes people that were retired from work. . With regard to occupation level, it was observed that the proportion of respondents who were employed for wages was the highest followed by the students, self-employed individuals, retired and, unemployed individuals had the least proportion.

Table 4: Frequency distribution of participants by age category, gender and occupation status

Variable type	Variable category	Number of respondents (%)
Age category (years)	Youth	254 (42.3)
	Adult	229 (38.2)
	Elderly	117 (19.5)
Sex	Male	304 (50.7)
	Female	296 (49.3)
Occupation level	Employed for wages	196 (32.7)
	Not employed	32 (5.3)
	Self, employed	137 (22.8)
	Retired	70 (11.7)
	Students	165 (27.5)

4.3 Concentration of aacrylamide in ready-to-eat potato chips

In the present study, two different types of ready -to-eat potato chips were analysed for determination of acrylamide content (n=100). Results of acrylamide content in the two types of ready-to-eat potato chips are presented in Table 6. Overall the findings of this study indicated that acrylamide levels in ready-to-eat potato chips ranged from 103μg/kg to 1056µg/kg, with an average value of 326.92µg/kg. Potato chips mixed with eggs had had significantly higher levels acrylamide levels than plain potato chips ($P < 0.05$).

Regarding concentration of acrylamide in the chips based on the source from which the samples were collected, the findings showed that potato chips collected at hotels had the highest mean acrylamide levels followed by those collected at chips kiosk and chips collected at restaurant had the least mean acrylamide levels although the differences in acrylamide levels did not attain statistical significance. ($P > 0.05$).

Table 5: Acrylamide concentration in ready-to-eat potato chips samples from Kinondoni Municipality grouped according to type and source of sample

Variable	Category	Number of samples (n)	Acrylamide concentration (µg/kg)				P value
			Mean ± SD	95 % CI	Min	Max	
Chips type	Plain chips	50	282.78 ± 169.17	(235.70, 330.86)	103	981	0.010
	Chips mixed with egss	50	371.06 ± 167.86	(323.36, 418.76)	142	1056	
Source of sample	Chips kiosk	92	328.01 ± 178.48	(291.46, 364.56)	112	1056	0.420
	Restaurant	4	234.25 ± 94.90	(83.24, 385.26)	103	323	
	Hotel	4	394.50 ± 145.65	(162.74, 626.26)	280	600	
	All samples	**100**	**326.92 ± 173.43**	**(292.51, 361.33)**	**103**	**1056**	

CI= confidence interval, Min= Minimum and Max = Maximum

4.4 Exposure assessment

4.4.1 Consumption of ready-to-eat potato chips

Estimated *per capita* consumption of ready-to-eat potato chips for the respondents based on their age groups, gender and occupation status are presented in Table 5. Overall, the findings showed that the mean *per capita* consumption for Kinondoni Municipality's residents aged between 15 to 70 years ranged between was estimated to be 170. 98 g/person/day.

Looking at individual age groups, the findings revealed that *per capita* consumption of ready-to-eat potato chips for all types of potato chips were significantly different ($P < 0.05$). In terms of consumption of the chips based on gender, the findings of this study showed that the differences in mean consumption of ready-to-eat potato chips between males and females were insignificant. ($P > 0.05$). When consumption of ready-to-eat potato chips was compared based on occupation of the respondents, it was demonstrated that there were significant difference in consumption of the chips across different occupational groups ($P < 0.05$).

Table 6: Estimated *per capita* consumption of ready-to-eat potato chips (g/person/day) for the respondents based on their age groups, gender and occupation status

Variable	Category	Ready-to-eat plain potato chips			Ready-to-eat chips mixed with eggs			Both ready-to-eat plain chips and chips mixed with eggs		
		Mean ± SD	P95	P value	Mean ± SD	P95	P value	Mean ± SD	P95	P value
Age group	Youth	97.12 ± 56.81	182	<0.001	102.47 ± 56.97	216	<0.001	199.59 ± 79.27	307	<0.001
	Adults	81.12 ± 53.72	182		93.11 ± 57.71	216		174.23 ± 72.93	306	
	Elderly	57.41 ± 36.06	104		45.10 ± 29.01	108		102.51 ± 44.42	199	
Gender	Male	78.81 ± 51.01	182	0.046	88.19 ± 60.19	216	0.042	167.00 ± 75.69	306	0.227
	Female	87.61 ± 56.68	182		87.24 ± 53.80	216		174.86 ± 83.07	306	
Occupation	Employed	78.11 ± 51.56	182	< 0.001	93.67 ± 57.57	216	< 0.001	171.78 ± 72.36	306	< 0.001
	Self employed	78.77 ± 54.49	182		82.55 ± 60.32	216		161.33 ± 84.82	306	
	Not employed	88.28 ± 59.49	182		92.09 ± 45.45	216		180.37 ± 75.96	306	
	Retired	55.72 ± 34.50	104		49.81 ± 33.76	108		105.79 ± 45.93	199	
	Student	103.75 ± 55.72	182		100.15 ± 56.57	216		203.89 ± 77.08	398	
All subjects		83.27 ± 54.10	182	NA	87.71 ± 56.99	216	NA	170.98 ± 79.68	306	NA

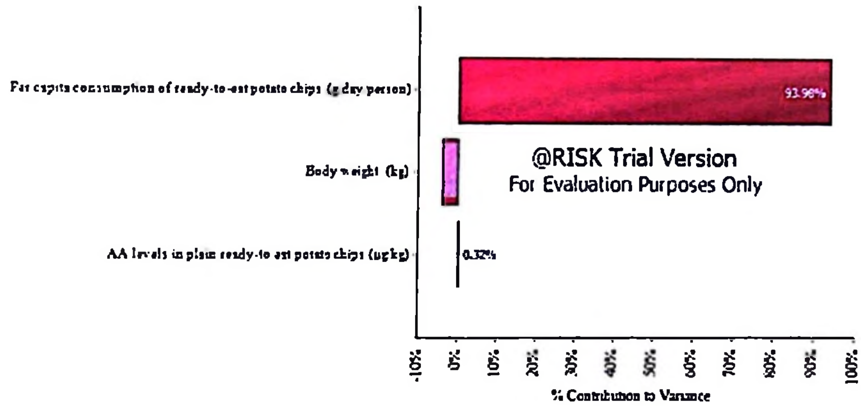
4.4.2 Dietary intake of acrylamide through consumption of ready-to-eat potato chips

The Monte Carlo simulation method was used to estimate population exposure distributions by fitting the suitable distribution of the input variables in the dietary acrylamide exposure equation. Probabilistic distributions for the mean and 95th percentile exposure to acrylamide are presented in Table 7. Furthermore, sensitivity analysis was conducted to evaluate contribution of each input variable on exposure to acrylamide for consumers. The outputs of sensitivity analysis are presented in Figures 2a, 2b, 2c and 2d. The findings of this study demonstrated that the mean and 95th percentile acrylamide exposure through consumption of ready-to-eat potato chips estimated in the current study was 0.179 µg/kg bw/day and 1.56 µg/kg bw/day, respectively. In addition, the findings indicated that, *per capita* consumption of ready-to-eat potato chips had highest contribution for dietary exposure to acrylamide among all the three input variables.

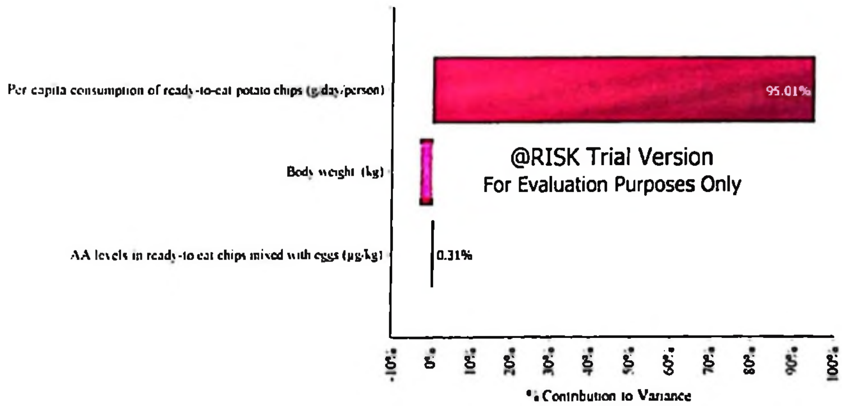
Table 7: Estimated exposure to acrylamide in µg/kg bw/day trough consumption of ready-to-eat plain ready-to-eat potato chips, chips mixed with eggs and total exposure in different age groups

Age group	Source of exposure					
	Plain ready-to-eat potato chips		Ready-to-eat chips mixed with eggs		Total exposure	
	Mean	P95	Mean	P95	Mean	P95
Youth	0.44	0.95	0.60	1.43	1.35	2.10
Adults	0.32	0.72	0.46	1.14	1.11	1.52
Elderly	0.19	0.43	0.19	0.49	0.42	0.73
All subjects	0.34	0.97	0.45	0.18	0.79	1.56

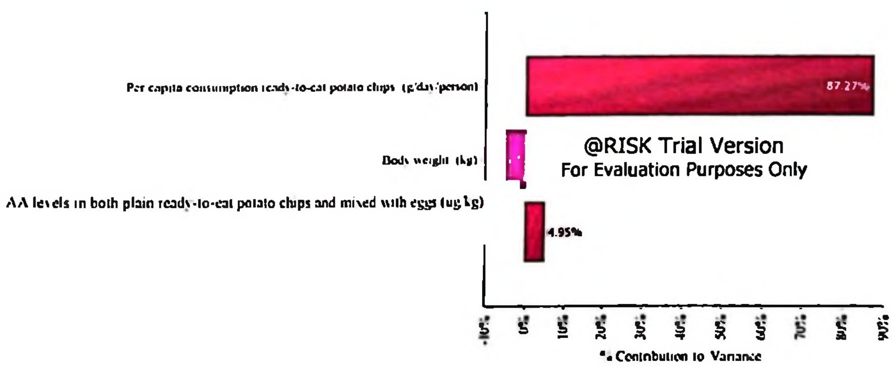
P95= 95th percentile



(a) Exposure through consumption of plain ready-to-eat potato chips

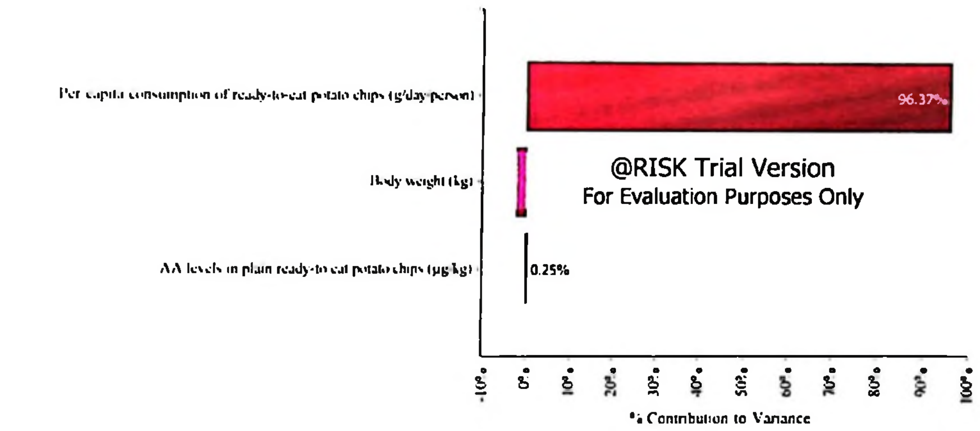


(b) Exposure through consumption of ready-to-eat chips mixed with eggs

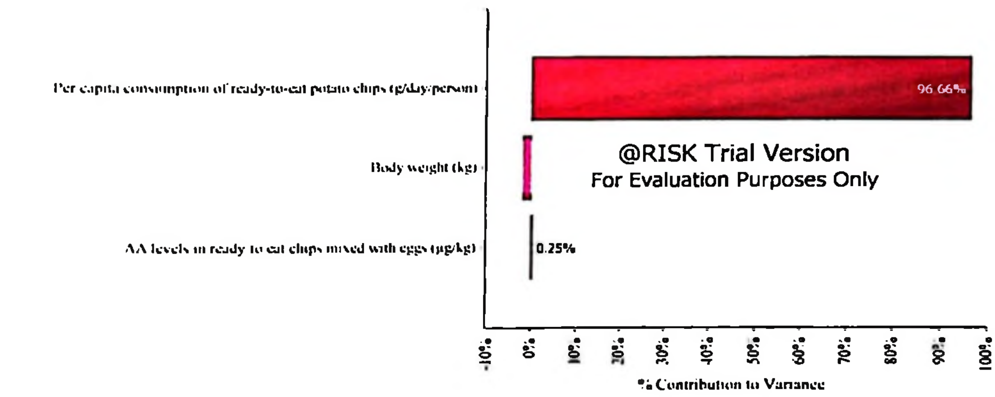


(c) Exposure through consumption of both plain ready-to-eat potato chips and potato chips mixed with eggs

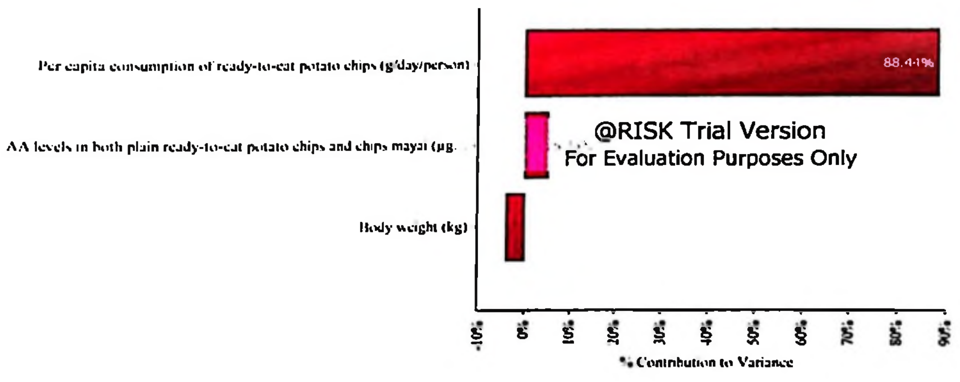
Figure 2a: Output for sensitivity analysis on the effect of input variables to acrylamide exposure for youths



(a) Exposure through consumption of plain ready-to-eat potato chips

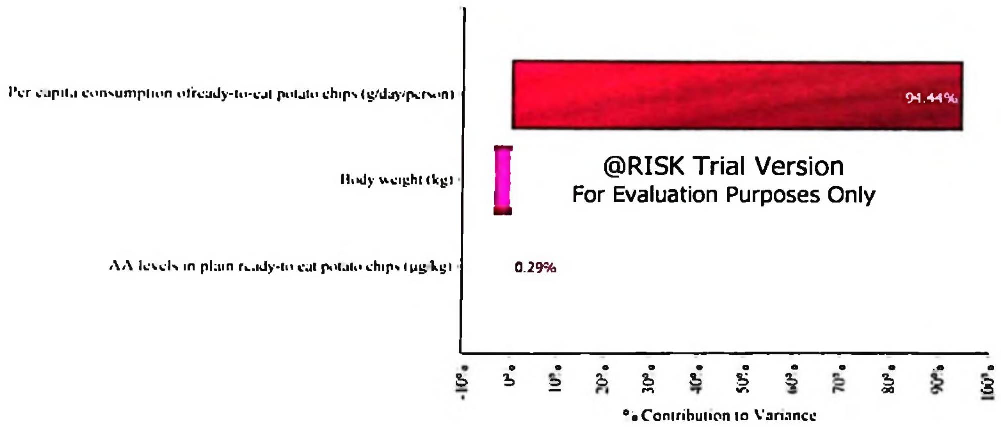


(b) Exposure through consumption of ready-to-eat potato chips mixed with eggs

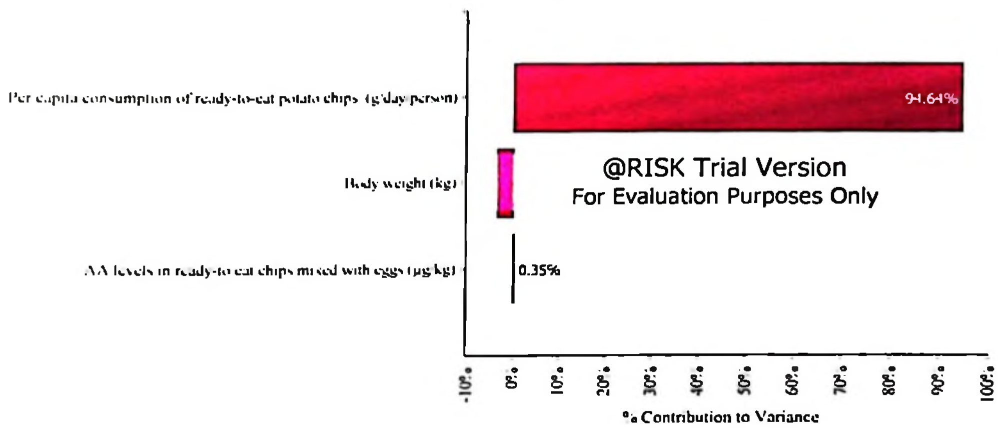


(c) Exposure through consumption of both plain ready-to-eat potato chips and potato chips mixed with eggs

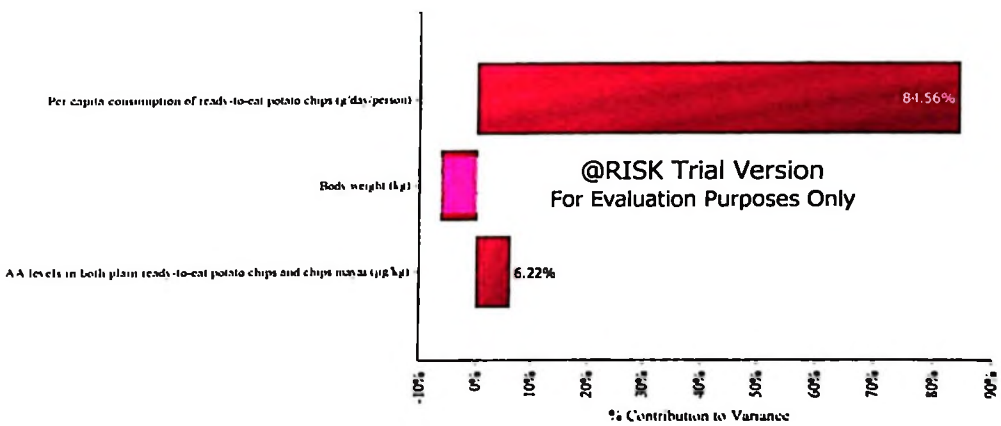
Figure 2b: Output for sensitivity analysis on the effect of input variables to acrylamide exposure for adults



(a) Exposure through consumption of plain ready-to-eat potato chips

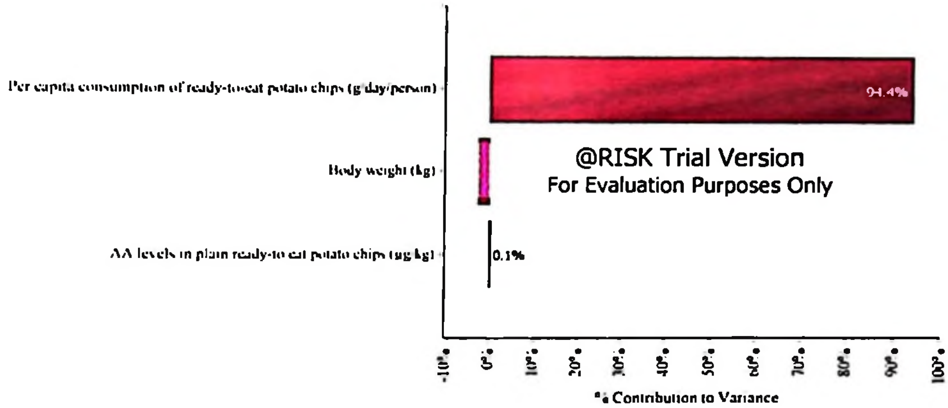


(b) Exposure through consumption of ready-to-eat potato chips mixed with eggs

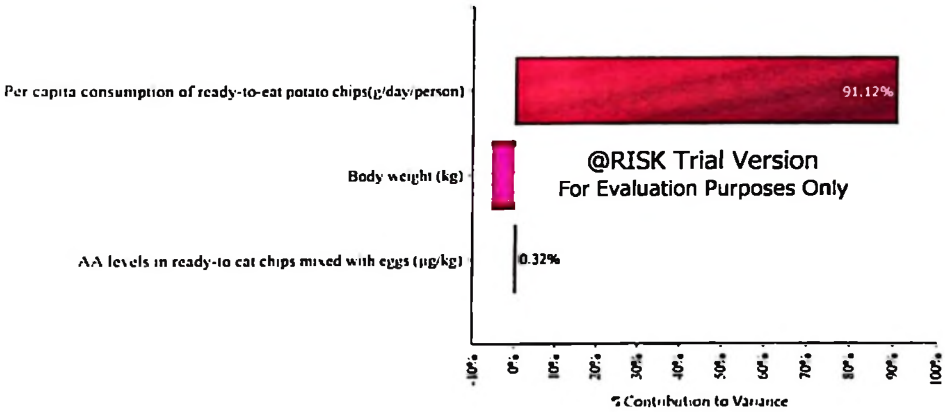


(c) Exposure through consumption of both plain ready-to-eat potato chips and potato chips mixed with eggs

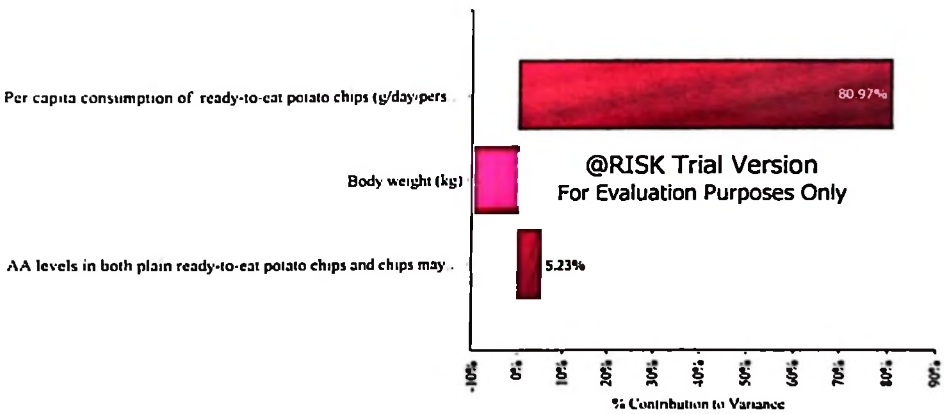
Figure 2c: Output for sensitivity analysis on the effect of input variables to acrylamide exposure for the elderly



(a) Exposure through consumption of plain ready-to-eat potato chips



(b) Exposure through consumption of ready-to-eat potato chips mixed with eggs



(c) Exposure through consumption of both plain ready-to-eat potato chips and potato chips mixed with eggs

Figure 2d: Output for sensitivity analysis on the effect of input variables to acrylamide exposure for all age groups

4.4.3 Risk characterization

The risk associated with exposure to acrylamide through consumption of ready-to-eat potato chips was estimated using the MOE approach based on (1) No Observed Adverse Effect Level (NOAEL) of 200 µg/kg bw/day based on morphological changes in rats; (2) $BMDL_{10}$ of 180 mg/kg bw/day derived by JECFA as the lowest $BMDL_{10}$ from data on incidences of Haderian gland tumours in male mice exposed to acrylamide for two years. MOE values for mean and higher consumers (95th percentile) associated with exposure for different varieties of ready-to-eat potato chips are presented in Figures 3a and 3b.

The findings in Fig. 3a and Fig. 3b demonstrate that MOEs values for mean and high exposure consumer calculated based on the NOAEL ranged from 134 to 1053 and 95 to 465, respectively. MOEs calculated based on the BMDL for mean and higher exposure consumers ranged from 180 to 1000 and from 77 to 346, respectively.

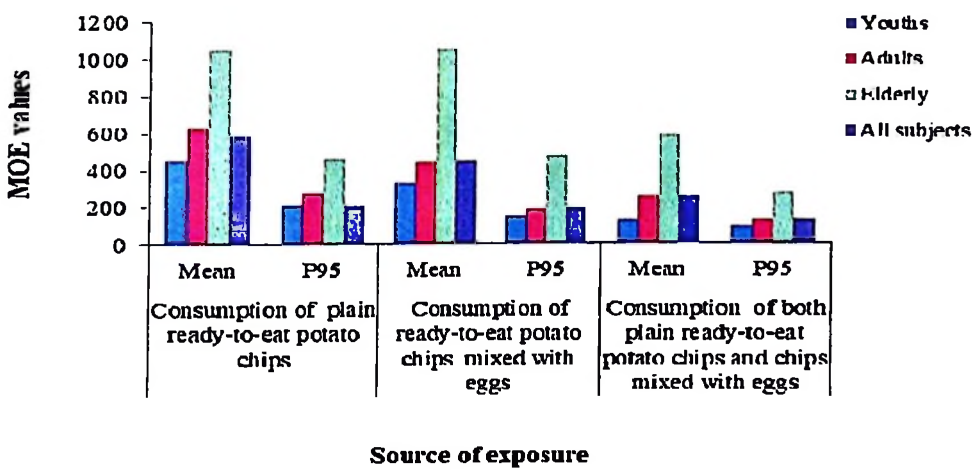


Figure 3(a): Margin of exposure between dietary exposure to acrylamide calculated based on NOAEL of 200 µg/kg bw/day

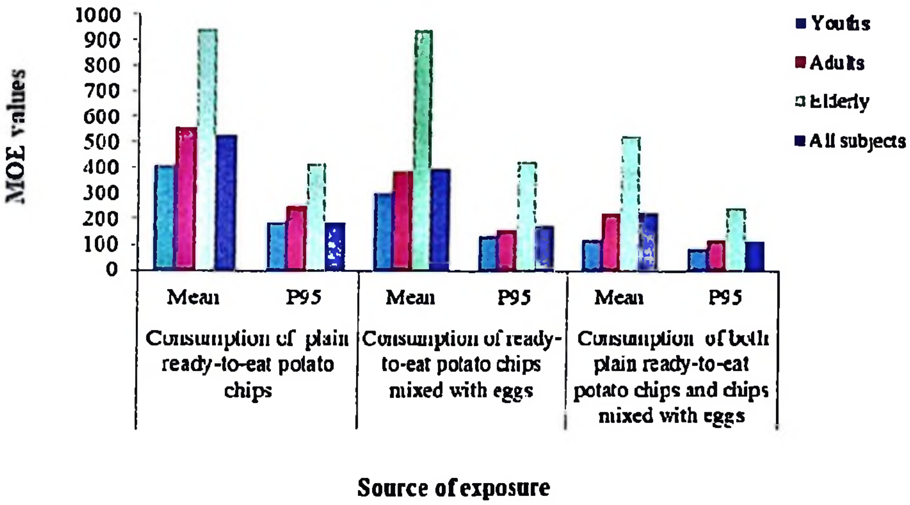


Figure 3(b): Margin of exposure d between dietary exposure to acrylamide calculated based on $MBDL_{10}$ of 200 µg/kg bw/day

CHAPTER FIVE

5.0 DISCUSSION

The current study was conducted in order to assess health risks associated with exposure to acrylamide through consumption of ready to-eat-potato chips in Kinondoni Municipality based on FAO/WHO guidelines. Dietary exposure to acrylamide trough consumption of ready-to-eat potato chips has been estimated by combing potato chips consumption data with data on acrylamide concentration in ready-to-eat potato chips using Monte Carlo simulation approach. Risk characterization has been estimated using MOE approach. It was general estimated that the mean and 95th percentile acrylamide exposure was 0.79μg/kg bw/day and 1.56 μg/kg bw/day, respectively.

Based on the benchmark dose lower confidence limit at 10% risk (BMDL10) of 0.31 mg kg_1 bw day_1 for the induction of mammary tumors in rats and 0.18 mg kg_1 bw day_1 for Harderian gland tumors in mice, the margins of exposure (MOEs) were and 180 and 77, respectively which indicates a human health concern.

5.1 Levels of acrylamide in ready-to-eat potato chips

The study analysed samples of ready-to-eat potato chips for acrylamide in order to determine the concentration of acrylamide in ready-to-eat potato. Acrylamide levels in ready-to-eat potato chips determined in this study ranged from 103 μg/kg to 1056 μg/kg with the mean value of 326.92 μg/kg depending on the type of potato chips. The mean acrylamide content obtained in this study are in the same range with the values published by JECFA (2011) and EFSA (2012) where in both publication mean acrylamide levels in potato chips ranged between 169 – 963 μg/kg.

In addition, the study established that acrylamide levels in ready-to-eat potato chips mixed with eggs were significantly higher as compared with the levels in plain ready-to-eat potato chips. Several research articles have demonstrated that the amount of acrylamide formed in potatoes during frying increased significantly with increase in temperature and time (Gertz et al, 2002; Pedreschi et al, 2005). Similar reasons may have also accounted for the higher levels of acrylamide in ready-to-eat potato chips mixed with eggs observed in the current study because preparation of ready-to-eat potato chips mixed with eggs involve more heat treatment and time than plain ready-to-eat potato chips. This result should be interpreted with caution because a study conducted by Fadwal et al (2009), revealed that higher temperature (200 degrees C) combined with prolonged heating times reduced levels of acrylamide.

Furthermore, when the results were presented based on the source from which the samples of ready-to-eat potato chips were collected (Table 5), there did appear to be a difference in acrylamide in ready-to-eat potato chips collected at chips kiosks, restaurants as well as hotels. Although this difference did not attain statistical significance ($P> 0.05$).

5.2 Health risks associated with acrylamide exposure via consumption of ready-to-eat potato chips

5.2.1 Consumption pattern of ready-to-eat potato chips

Per capita consumption of ready-to-eat potato chips is a very important input variable for estimation of human exposure to acrylamide through consumption of potato chips. Generally, mean *per capita* consumption of ready-to-eat potato chips in the current study estimated to be 170.98 g/person/day. The mean *per capital* consumption estimated in this study was not consistent with the values published at international levels. The World Health Organization of the United Nations in 2003 published a lower mean *per capita* consumption of ready-to-eat potato chips (20.6 g/person/day) for Africans (WH0, 2003) as compared to those estimated in the current study. From the same source a higher mean *per capita* consumption of the chips (240.8 g/person/day) was reported for the Europeans. This disagreement on mean *per capita* consumption data estimated in the current study and those published at international level can be attributed to smaller sample size used in the current study.

It was further observed that the highest mean *per capita* consumption of ready-to-eat potato chips was for youth while elderly had the lowest mean *per capita* consumption of ready-to-eat plain potato chips. The difference in mean *per capita* consumption across the different age groups was significant ($P < 0.05$) and that mean *per capita* consumption decreased from the youth to adults and elderly in that order. This implies that on average, youth consume the highest amount of ready-to-eat potato chips per day as compared to other category. A study carried out by Untaru *et al* (2013) ported that youth preference to fast foods were influenced by their low income status, lack of time, convenience and need for socialization. The same factors may also be linked with the highest consumption of ready-to eat- potato chips among youths in the study area.

Considering different genders, the result of this study show that the difference in mean *per capita* consumption of both plain ready-to-eat plain potato chips and ready-to-eat potato chips mixed with eggs were significant ($P < 0.05$). In terms of different occupation levels, the results in Table 5 reveals that mean *per capita* consumption of ready-to-eat plain potato chips of the mentioned groups demonstrated to have the significant difference ($P < 0.001$) in which the students showed the highest mean *per capita* consumption of all the other groups.

5.2.2 Exposure to acrylamide through consumption of ready-to-eat potato chips

This study estimated acrylamide exposure from 600 individuals in the study area covering the following age groups: youth (15 to 34 years), adults (35 to 54 years) and elderly (55 to 70 years). Overall mean acrylamide exposure for average and higher consumers estimated in this study were 0.79 µg/kg bw/day and 1.56 µg/kg bw/day, respectively. Both the average and high consumers' exposures estimated in this study fell above the range of dietary exposure of acrylamide in western countries (i.e. 0.3 to 0.8 µg/kg body weight/day) as estimated by the WHO at the expert consultation meeting in June 2002 (FAO/WHO, 2002). The estimated mean dietary exposure was 0.5 µg/kg body weight/day and 1.5 µg/kg body weight/day for high consumers in Australia in 2004. For New Zealand, the 2006 exposure data indicated a dietary exposure between 0.9 to 2.4 µg/kg body weight/day. For United States, the Food and Drug Administration estimated the mean exposure level in 2006 to be 0.4 µg/kg body weight/day18, while Health Canada reported in 2009 that the estimated mean exposure level for Canadian adults to be between 0.3 and 0.4 µg/kg body weight/day. The European Food Safety Authority estimated that the mean exposure level for EU member states was 20 µg/day in 2007 and 30 µg/day in 2003 – 2006 respectively. Therefore, the local mean dietary intake of acrylamide was not particularly high when compared with the above countries.

5.2.3 Risk characterization

The use of MOE approach for risk characterization for substances that are both genotoxic and carcinogenic provides advice to inform risk managers how close human exposures are to those anticipated to produce an adverse effect. Since acrylamide is one of the genotoxic and carcinogenic compound, in the current study risk characterization was estimated using MOE approached based on Based on the benchmark dose lower confidence limit at 10%

risk (BMDL10) of 0.31 mg kg_1 bw day_1 for the induction of mammary tumors in rats and 0.18 mg kg_1 bw day_1 for Harderian gland tumors in mice.

The findings of this study demonstrated that, MOE values estimated based on NOEL, ranged from 134 to 1053 and 95 to 465 for mean and high exposed individuals, respectively. Furthermore, the findings revels that MOE estimated based on BMDL for mean and higher exposed individuals ranged from 180 to 1000 and from 77 to 346, respectively. In both cases, the estimated MOEs values estimated in the current study were substantially lower than 10,000, indicating a health concern for individual with the age between 15 to 70 years at Kinondoni Municipality.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6 .1 Conclusions

This is the first study to be conducted in Tanzania to assess health risks due to exposure to acrylamide through consumption of ready-to-eat potato chips. Based on the findings of this study it can be concluded that:

i. The levels of acrylamide in ready-to-eat potato chips are in the same range as the values published by JECFA and WHO.

ii. Potato chips mixed with eggs had significantly higher levels acrylamide as compared with plain ready-to-eat potato chips.

iii. Estimated mean *per capita* consumption of ready-to-eat potato chips was not consistent with mean *per capita* consumption of potato chips for Africans and Europeans as established by the JECFA.

iv. Dietary intakes of acrylamide via consumption of ready-to-eat potato chips for Kinondoni municipality resident are in line with those values published by JECFA recently.

v. The MOE values obtained in this study show that exposure to acrylamide due to consumption of ready-to-eat potato chips indicates a human health concern.

5.2 Recommendations

From the conclusion drawn, it is therefore recommended that:-

i. Education about reduction of acrylamide formation in ready-to-eat potato chips during processing is an important measure to prevent acrylamide health risks. Potato chips vendors in Kindondoni Municipality and Tanzania at lager should be offered education on appropriate methods to reduce the level of acrylamide in potato chips to as low as reasonably possible.

ii. There is a need to establish monitoring and surveillance program on concentration of acrylamide foods.

iii. Research effort should be directed towards conducting a total diet study so as to establish the most accurate acrylamide dietary exposure to the consumers.

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APPENDICES

Appendix 1: Food frequency questionnaire on ready-to-eat potato chips consumption (English version)

<u>INTRODUCTION</u>

I am John Mwingira, a student from the Sokoine University of Agriculture (SUA). I am currently doing my research which requires to be completed for me to be able to graduate with Msc. Epidemiology. My research title is "*Health Risk Assessment due to exposure to acrylamide through consumption of potato chips in Kinondoni Municipality*".

The purpose of this interview is to capture information on consumption of potato chips. This information will be useful for assessment of health risk due *to exposure to acrylamide* through consumption of potato chips.

I am inviting you to take part in this research project. If you accept, you will be interviewed on consumption of potato chips. Your participation in this research is entirely voluntary. It is your choice whether to participate or not. During the interview, I or another interviewer will sit down with you in a comfortable place. If you do not wish to answer any of the questions during the interview, you may say so and the interviewer will move on to the next question. The interview will be recorded in a questionnaire. No one else but the interviewer will be present unless you would like someone else to be there. Your name will not be written in the questionnaire. The information recorded is confidential, and no one else except researchers will be able to access the information documented during your interview. We will not be sharing information about you to anyone outside of the research team.

The findings of this study will have no direct effect to you instead, may be usefully to food regulatory authorities such as Tanzania Food and Drugs Authority and Tanzania Bureau of Standards in designing intervention strategies minimize the acrylamide intake from potato chips.

This consent form may contain words that you do not understand. Please ask me to stop as we go through the information and I will take time to explain. If you have questions later, you can ask me or another researcher.

Questionnaire No:__________

Date of interview: (dd/mm/yyyy):___/___/___

Ward:______________________________

Street:______________________________

1.0 Personal information

1.1 Sex of respondent (Please tick one)

a) Male

b) Female

1.2 Age of respondent (years):________

1.3 Employment status of respondents. (Please tick one)

a) Employed for wages

b) Self employed

c) Not employed

d) Retired

e) Student

1.4 Weight of respondent (kg):__________

2.0 Information about consumption of ready-to-eat potato chips

2.1 (a) Do you consume plain ready-to-eat potato chips? (Please tick one)

i) Yes (If yes go to 2.1 (b)

ii) No (If no go to 2.2 (a)

(b) How often do you eat plain ready-to-eat potato chips per week? (Please tick one)

i) Every day

ii) After every other day

iii) Once per week

iv) Twice per week

v) Others; specify____________

(c) Each time you eat plain ready-to-eat potato chips, how many plates do you usually eat per day? (Please tick one)

Number of plates per day						
1	2	3	4	5	6	>6

2.2 (a) Do you consume ready-to-eat chips mixed with eggs? (Please tick one)

iii) Yes (If yes go to 2.2 (b)

iv) No (If No go to 2.3)

(b) How often do you eat ready-to-eat chips mixed with eggs per week? (Please tick one)

vi) Every day

vii) After every other day

viii) Once per week

ix) Twice per week

x) Others; specify_____________

(c) Each time you eat ready-to-eat chips mixed with eggs, how many plates do you usually eat per day? (Please tick one)

Number of plates per day						
1	2	3	4	5	6	>6

2.3 Where do you source ready-to-eat potato chips?

i) Chips vendor

ii) Restaurant

iii) Hotel

iv) At home

v) Others; Specify:_______________

"THANK YOU VERY MUCH FOR YOUR TIME AND GOOD COOPERATION"

Appendix 2: Appendix Food frequency questionnaire on ready-to-eat potato chips consumption (Swahili version)

<u>UTANGULIZI</u>

Jina langu ni John Mwingira, mwanafunzi wa Chuo Kikuu cha Kilimo Sokoine. Nafanya utafiti amabao kukamilika kwake kutaniwezesha kuhitimu Shahada ya Uzamili ya Epedemiolojia. Utafafiti huu unahusu tathmini ya madhara ya kiafya yanayoweza kusababishwa na kemikali aina ya "*Acylamide*" kupitia ulaji wa chips.

Tunakuomba uwe mmoja wa washiriki watakaohojiwa. Endapo utaridhia utahojiwa kuhusu kuhusu ulaji wa "chips". Lengo la mahojiano hayo ni kupata taarifa kuhusu ulaji wa chips. Taarifa hiyo ni muhimu katika kufanya tathmini ya madhara ya kiafya yanayoweza kusabishwa na kemikali aina ya *acylamide* kupitia ulaji wa chips. Utahojiwa na wahojaji katika mazingira ambayo wewe unajisikia amani na huru. Kama hautajisikia kujibu swali lolote kati ya maswali utakayoulizwa usisite kusema hivyo ili muhojaji aendelee kukuhoji maswali mengine. Majibu yako yataandikwa katika karatasi yenye maswali (dodoso) bila kuweka jina lako. Taarifa utakazozitoa ni siri na hakuna mtu mwingine yeyote zaidi ya timu ya watafiti ambaye anaweza kujua majibu uliyoyatoa. Ushiriki wako kwenye utafiti huu ni wa hiari.

Utafiti huu hautakuwa na manufaa ya moja kwa moja kwako kwa sasa, lakini utasaidia kuimarisha udhibiti wa kemikali hii hapa Tanzania na kusaidia ulinda afya ya walaji.

Endapo una swali au kutaka ufafanuzi wowote kuhusu utafiti huu usisite kuuuliza kwa mhojaji.

Namba ya dodoso:________

Taarehe ya kuhojiwa (tarehe/mwezi/mwaaka) __/___/____

Kata: ____

Mtaa:____

1.0 Taarifa binafsi

1.1 Jinsia ya mhojiwa (Tafadhali weka alama ya vema kwenye eneo linalohusika)

i) Mwanaume

ii) Mwanamke

1.2 Umri wa mhojiwa (miaka): ___________

1.3 Hali ya ajira ya mhojiwa? (Tafadhali weka alama ya vema kwenye eneo linalohusika)

i) Nimeaajiriwa

ii) Nimejiajiri mwenyewe

iii) Sina kazi

iv) Nimestaafu

v) Mwanafunzi

1.4 Uzito wa mhojiwa (kg):________

2.0 Taarifa kuhusu ulaji wa chips

2.1 (a) Uwa unakula chips kavu? (Tafadhali weka alama ya vema kwenye eneo linalohusika)

i) Ndiyo (Kama jibu ni Ndiyo nenda swali Na. 2.1 (b)

ii) Hapana (Kama jibu ni Hapana nenda swali Na. 2.2 (a)

(b) Kwa kawaida ni mara ngapi huwa unakula chips kavu ndani ya wiki moja? (Tafadhali weka alama ya vema kwenye eneo linalohusika)

i) Kila siku

ii) Kila baada ya siku moja

iii) Mara moja kwa juma

iv) Maara mbili kwa juma

v) Nyingine; (Eleza)...

(c) Kila wakati unapokula "chips" kavu, ni sahani ngapi kwa wastani huwa unakula kwa siku? (Tafadhali weka alama ya vema kwenye eneo linalohusika)

Idadi ya sahanni kwa siku						
1	2	3	4	5	6	>6

2.2 (a) Huwa unakula chips-mayai? (Tafadhali weka alama ya vema kwenye eneo linalohusika)

iii) Ndiyo (Kama jibu ni Ndiyo nenda swali Na. 2.2 (b)

iv) Hapana (Kama jibu ni Hapana nenda swali Na. 2.3

(d) Kwa kawaida ni mara ngapi huwa unakula chips-mayai ndani ya wiki moja? (Tafadhali weka alama ya vema kwenye eneo linalohusika)

vi) Kila siku

vii) Kila baada ya siku moja

viii) Mara moja kwa juma

ix) Maara mbili kwa juma

x) Nyingine; (Eleza)..

(e) Kila wakati unapokula "chips"-mayai, ni sahani ngapi kwa wastani huwa unakula kwa siku? (Tafadhali weka alama ya vema kwenye eneo linalohusika)

Idadi ya sahanni kwa siku						
1	2	3	4	5	6	>6

2.3 Ni wapi huwa unapata chips?

i) Kibanda cha "chips"

ii) Kwenye mgahawa

iii) Hotelini

iv) Nyumbani

v) Nyingine; (Elezea)_______________

"ASANTE KWA USHIRIKIANO ULIOONESHA"

Appendix 3 Sample collection form

1. Ward: ....
2. Date of sample collection: dd/mm/yyyy): ....../....../............
3. Sample Identification Number: ....
4. Source of sample (please tick one)
   a. Hotel ☐ c. Restaurant ☐
   b. Food vendors ☐ d. Others; Specify: ....
5. Chips variety (Please tick one)
   a. Ready-to-eat plain potato chips ☐
   b. Ready-to-eat *chips-mayai* ☐
6. Weight of the sample (gm):______ (To be weighed in the laboratory using weigh scale)
7. Acrylamide level (μg/kg):______ (To be filled after laboratory analysis)

Appendix 4: A research introductory letter from Sokoine University of Agriculture

KIBALI CHA KUFANYA UTAFITI NCHINI TANZANIA

CHUO KIKUU CHA SOKOINE CHA KILIMO
OFISI YA MAKAMU WA MKUU WA CHUO
S.L.P. 3000, MOROGORO, TANZANIA
Simu:023-2604523/2603511-4; Fax:023-2604651, MOROGORO

Kumb. Zetu : SUA/ADM/R.1/8 Tarehe 8 Oktoba, 2015

Mkurugenzi wa Manispaa
Halmashauri ya Manispaa ya Kinondoni
KINONDONI

UTAFITI WA WAALIMU NA WANAFUNZI WA CHUO KIKUU

Madhumuni ya barua hii ni kumtambulisha kwako John Mwingira ambae ni Mwanafunzi wa Uzamili katika Chuo Kikuu cha Sokoine cha Kilimo (SUA). Huyo ndugu hivi sasa yuko katika shughuli za utafiti.

Chuo Kikuu cha Sokoine cha Kilimo (SUA) kilianzishwa na Sheria ("Universities Act No.5 of 2005") na Hati Idhini ("SUA Charter, 2007") ambayo ilianza kutumika Januari 1, 2007. Hati Idhini ilichukua nafasi ya Sheria Na.6 ya mwaka 1984. Moja ya majukumu ya SUA ni kufanya tafiti mbalimbali na kutumia matokeo ya tafiti hizo. Kwa sababu hiyo, waalimu, wanafunzi na watafiti wa Chuo hufanya tafiti mbalimbali katika nyakati zinazostahili.

Ili kufanikisha utekelezaji wa tafiti hizo Makamu wa Mkuu wa Chuo SUA amepewa mamlaka chini ya Hati Idhini ya SUA ya kutoa vibali vya kufanya utafiti nchini kwa waalimu, wanafunzi na watafiti wake.

Hivyo basi tunnomba umpatie mwanafunzi aliyetajwa hapo juu msaada atakaohitaji ili kufanikisha uchunguzi wake. Gharama za malazi na chakula chake pamoja na usafiri wake atalipia wenyewe kutokana na fedha alizopewa. Msaada anaouhitaji zaidi ni kuruhusiwa kuonana na viongozi na wananchi ili aweze kuzungumza nao na kuwauliza maswali aliyo nayo.

Kiini cha Utafiti wa mwanafunzi aliyetajwa hapo juu ni: "Kufanya tathmini ya madhara ya kiafya yanayoweza kusababishwa na kemikali aina ya *Acylamide* kupitia ulaji wa chipsi".

Sehemu anayofanyia utafiti huo ni Manispaa ya Kinondoni. Ikiwa kuna baadhi ya sehemu ambazo zinazuiliwa, ni wajibu wako kuzuia zisitembelewe.

Muda wa Utafiti huo ni kuanzia tarehe 1.11.2015 hadi 28.2.2016.

Wasalaam,

Prof. Gerald C. Monela
MAKAMU WA MKUU WA CHUO

MAKAMU WA MKUU WA CHUO
CHUO KIKUU CHA SOKOINE CHA KILIMO
S. L. P. 3000
MOROGORO, TANZANIA

Nakala: Mtafiti

VICE CH. R
SOKOINE UNIVERSIT ULTURE
P. O. Box 3
MOROGORO, TAN

Appendix 5: A research permission letter from Kinondoni Municipal Council

KINONDONI MUNICIPAL COUNCIL

ALL CORRESPONDENCES TO BE ADDRESSED TO THE MUNICIPAL DIRECTOR

Tel. 2170173
Fax: 2172606

In reply please quote:

Ref. KMC/F.6/5

MUNICIPAL DIRECTOR
KINONDONI MUNICIPAL COUNCIL
P. O. BOX 31902
2MOROGORO ROAD
14883 DAR ES SALAAM

Date 15/12/2015

John Mwingira,
Sokoine University of Agriculture,
P. O. Box 3000,
MOROGORO.

RE: RESEARCH ATTACHMENT

Refer to the above heading.

I am pleased to inform you that your above request has been considered by the Municipal Director, and has offered you a place to conduct research attachment from **01 November,2015 to 28 February,2016.**

Upon receipt of this letter, please report to the **Ward Executive Officer – Magomeni Makurumla,Ndugumbi,Tandale,Goba,Mbezi Juu,Ubungo Wards** for commencement of your research. -

During the period of field you are required to obey the rules and regulations of the institution as they will be defined by the supervisor of the research.

Hoping to see you soon.

for: MUNICIPAL DIRECTOR
KINONDONI MUNICIPAL COUNCIL
DAR ES SALAAM

J. H. Alute
For: THE MUNICIPAL DIRECTOR
KINONDONI

Copy : Vice Chancellor,
Sokoine University of Agriculture.
P. O. Box 3000,
MOROGORO.