ACRYLAMIDE CONTAMINATION IN COMMERCIAL POTATO CRISPS IN KENYA: LEVELS OF INTAKE AND EFFECTS OF PROCESSING PARAMETERS IN LOCAL CULTIVARS

JACKLINE AKINYI OGOLLA

(B.Sc. Food Science and Technology, (UoN))

A dissertation submitted in partial fulfillment of the requirements for the award of the Degree of Master of Science in Food Safety and Quality in the Department of Food Science, Nutrition and Technology of the University of Nairobi

JULY, 2013

DECLARATION

This dissertation is my original work and has not been presented for a degree in any other university.

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

Ogolla Akinyi Jackline.

This dissertation has been submitted with our approval as University Supervisors:

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

Prof. Michael W. Okoth

Department of Food Science, Nutrition and Technology.

University of Nairobi

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

Dr. George O. Abong'

Department of Food Science, Nutrition and Technology.

University of Nairobi

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

Dr. Jackson N. Kabira

National Potato Research Centre

Kenya Agriculture Research Institute- Tigoni

DEDICATION

I dedicate this work to my late parents Dr. Alex Ogolla and Rose Adhiambo Ogolla, for the great and solid foundation they gave me in my formative years of life.

To my sister Celline who had to put on hold her ambitions at a tender age to ensure that we completed our studies after the demise of our parents. To my brothers Charles and Fredrick who are several standards above the normal mean and to my other lovely siblings: Evleen, Peter and Beatrice; who each bring an inspiration, encouragement and challenge to the table of this achievement.

.

ACKNOWLEDGEMENT

To God who always has a stake in any activity with a successful outcome, be all glory and honour. I glorify and praise the Lord God Almighty for the gift of life, His blessings, mercy, favour and grace which He has instilled upon me throughout the study period.

I am greatly indebted to my Supervisors, Prof. Michael. W. Okoth, Dr. George Ooko Abong' Department of Food Science, Nutrition and Technology and Dr. Jackson Kabira of the National potato research centre for the for their invaluable advice, guidance, encouragement, suggestions and constructive criticisms from the research proposal development to dissertation writing which have led to the successful completion of this study. Their contribution is highly treasured.

I wish to acknowledge the University of Nairobi for offering me a scholarship to pursue this course. I am also grateful to the University of Nairobi and National potato research centre (KARI) for financial support in undertaking this study. More appreciation goes to the Jomo Kenyatta University of Agriculture and Technology department of Food Science for support in laboratory analysis with greater appreciation to Mr. P.N. Karanja. The assistance offered by Mr. David, Mrs. Jessica, Mrs, Caroline and Mr. Waswa, Food Science and Technology Laboratory Technicians at the Jomo Kenyatta University of Agriculture and Technology is appreciated.

I wish to acknowledge all the individuals that contributed towards the successful completion of this work and whose constant encouragement and belief in me ensured that I finished successfully; Rose Gichure, Caroline Mwendwa, Immanuel Ekesa, Robert Cheruiyot, Rose Uzel, Chemutai Sawe, George Oketch, Dr. David Amala and Dr. Fred Obonyo.

Lastly I acknowledge my brothers Charles, Fred and Peter for the continuous encouragement and financial support towards the completion of this course.

I appreciate your commitment and may God bless you all.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

AA	Acrylamide
ALARA	As low as reasonably achievable
ANOVA	Analysis of variance
BC	Branded crisps
BMDL	Benchmark dose for a 10% response
bw	body weight
CI	Confidence interval
EFSA	European Food Safety Agency
EPA	Environmental Protection Agency
FAO	Food Agricultural Organisation
GC-MS	Gas Chromatography Mass Spectrophotometer
HPLC	High Performance Liquid chromatography
IARC	International Agency for Research on Cancer
IUPAC	International Union of Pure and Applied Chemistry
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KARI	Kenya Agricultural Research Institute

µg/kg	Micrograms per kilograms
MOE	Margins of exposure
ND	Non Detectable
NOAEL	No Observed Adverse Effect Level
SAS	Statistical Analysis System
SCNFCA	Scientific Committee of the Norwegian Food Control Authority
SPE	Solid Phase Extraction.
SVR	Surface Volume Ratio
UK	United Kingdom
USA	United States of America
WHO	World Health Organisation

General Abstract

The presence of acrylamide in several carbohydrates rich foods baked at high temperatures and its classification as a suspected human carcinogen calls for a concerted effort to minimize its presence in human diet. It is mainly formed in fried and baked carbohydrate rich foodstuffs such as potato chips and crisps through maillard reaction at elevated temperatures.

This study was designed in three phases with each phase covering a specific objective. The first part of the study involved a cross-sectional survey of commercial potato crisp outlets in Nairobi and laboratory analysis of the samples purchased. Both purposive and simple random sampling was used in choosing the districts to be surveyed. A total of 35 brands in duplicates were purchased from retail outlets in Nairobi and 15 unbranded samples from the street vendors within the five districts. The parameters analysed were moisture content, colour and acrylamide content. The second objective involved assessing the exposure to acrylamide through consumption of potato crisps in Nairobi, Kenya. Potato crisps consumption survey was carried out among crisps consumers. The data were collected from the consumers at the retail outlets where they bought the crisps. Consumption data were combined with contamination data arising from analysis of crisps from retail outlets and street processors in the same region and dietary acrylamide exposure was calculated using probabilistic approach. The third objective involved the determination of the effect of variety and processing conditions on crisps from local potato varieties. Four potato varieties; Tigoni, Kenya Mpya, Dutch Robjin and Sheherekea were planted under standard conditions in KARI, Tigoni. Harvesting was done at maturity and the tubers were sliced to three thicknesses of 1.0mm, 1.5mm and 2.0mm which were then each subjected to frying temperatures of 160°C, 170°C and 180°C. The raw tubers were analyzed for dry matter content and reducing sugars while the processed potato crisps were analyzed for colour, moisture, acrylamide and sensory properties.

Acrylamide levels significantly (P≤0.05) differed between the traded crisps brands ranging from non-detectable levels to 8666 µg kg^{-1} in the branded samples while in the unbranded samples it ranged from 5666 µg kg^{-1} in Vendor 7 to 9499 µg kg−1 in Vendor 6. There was a significant difference (P≤0.05) in acrylamide levels between the branded and the unbranded (street) potato crisps. The levels of acrylamide in the branded flavoured potato crisps ranged from non-detectable levels to 5151 µg kg^{-1}

The mean acrylamide intake was 1.57µg/kg bw/day while the 95th (P95) percentile was 5.1µg/kg bw/day with Margins of Exposures (MOE) being respectively 197 and 61. The acrylamide intake was significantly (P≤0.05) higher in street processed crisps (non-branded) with a mean value of 2.26 µg/kg bw/day and 95th percentile of 6.54 µg/kg bw/day, and MOE being respectively 137 and 47. The extremely lower MOEs exposure to acrylamide by the consumers could mainly be attributed to higher contamination levels.

Acrylamide levels significantly (P≤0.05) differed between the varieties and ranged from 13480 µg kg^{-1} in Kenya Mpya to Dutch Robjin recording the lowest levels of the acrylamide 3150 µg kg^{-1}. Acrylamide levels significantly increased with frying temperature and slice thickness. Important steps are therefore required to mitigate the high exposure by reducing level of acrylamide contamination and decreasing consumption. Appropriate varieties and processing parameters have to be chosen to ensure less acrylamide in potato crisps.

Key words: Acrylamide, reducing sugar, colour, thickness, temperature, variety, processing.

CHAPTER ONE

GENERAL INTRODUCTION

1.0 Background information

Potatoes (*Solanum tuberosum)* are high in carbohydrate and vitamin C, and are perceived by consumers as an important component of a healthy balanced diet. With a yearly worldwide production of approximately 330 million tons, the potato is thus one of the world's major staple food crops. Globally, potato is the fourth most widely grown food crop after rice, wheat and maize (MoA, 2007). Potato comes second as the most valuable cash and food crop after the cereal grains in Kenya according to the Ministry of Agriculture (MoA, 2009). Potato crisps and French fries are the most consumed potato products (Kulkarni et al., 1994; Knol et al., 2009). According to the East African Standards (2010) and Salvador *et al.(* 2008a), the definition of a potato crisp is given as a fragile but firm slice that has been processed through deep frying and edible salt or acceptable food grade spices colour and flavour may have been added.

Following the announcement by the University of Stockholm and Swedish National Food Authority in April 2002 on the presence of acrylamide majorly in carbohydrate rich foods, intensive investigations have been undertaken, involving the analysis, occurrence, chemistry, toxicology and potential health risk of this contaminant in the human diet (Tarek et al., 2002).

Acrylamide has been classified as a Group 2A carcinogen by the International Agency for Research on Cancer (IARC, 1994) and remains a suspected human carcinogen and a neurotoxicant that calls for a concerted effort to minimize its presence in all human diets (LoPachin, 2004; Stadler et al., 2004; Viklund et al., 2010). Acrylamide is mainly formed in fried and baked carbohydrate-rich foodstuffs such as potatoes as its formation pathway is linked with the reaction involving sugars and free amino acids at elevated temperature.

The estimated exposure to acrylamide has been shown to depend on dietary habits of consumers in different countries (Dybing et al., 2005). Consumption patterns vary among people of different ages, and younger people for instance, tend to eat more snacks than older people (Konings et al., 2003), which may result in a higher intake of acrylamide among the younger generation and hence higher potential risk of developing cancer related disorders. The consumption pattern is similar in Kenya and has been on the increase (Abong' et al., 2010). Crisps processed from potatoes have tremendously gained popularity especially in major towns in Kenya (Abong' et al., 2010). Most city dwellers consume potato crisps as snack between meals and during festivities.

The acrylamide content in fried potato products can be related to color (Pedreschi et al., 2005) and hence the need to monitor color parameters of acrylamide prone products such as potato crisps. Color is also an important quality parameter that determines the acceptance of fried potato products.

Since there is limited data on acrylamide content in potato crisps in the Kenyan market, this study aimed at evaluating the levels of acrylamide in commercial crisps in the Kenyan market and determining the relationship between color and acrylamide formation. In addition, the comparison of the acrylamide content in branded and in the unbranded potato crisps was done. The acquired information is useful in determining the level of exposure to acrylamide by potato crisps consumers.

1.1 Problem statement and Justification

Fried potato products including crisps have been reported to contain high levels of acrylamide, a potential carcinogenic substance of great concern not only to consumers, but also to the authorities and the food industry (Tareke et al., 2002: Viklund et al., 2008).

The estimated exposure to acrylamide has been shown to depend on dietary habits of consumers in different countries (Dybing et al., 2005). Svensson et al., (2003) reported a contribution of potato crisps to acrylamide intake in Sweden to be about 9% in consumers aged between 17 and 74 years. The contribution of the same snack in Netherlands was, however, reported to be quite high, 31% for consumers in the age bracket 1–97 years (Konings et al., 2003). Consumption patterns vary among people of different ages, and younger people for instance, tend to eat more snacks than older people (Konings et al., 2003), which may result in a higher intake of acrylamide among the younger generation and hence higher potential risk of developing cancer related disorders. The consumption pattern is similar in Kenya and has been on the increase (Abong' et al., 2010). The contribution of potato crisps in terms of acrylamide is, however, not supported by any data. Neither is there information on acrylamide content of the commercially sold potato crisps in Kenyan market nor acrylamide levels as affected by temperature and slice thickness in crisps from Kenyan potato varieties. The current study is designed to fill these knowledge gaps.

1.2 Study aim

To create awareness of acrylamide to the Kenyan population and ultimately improve on the pool of knowledge on acrylamide.

1.3 Purpose

To provide information on the levels of acrylamide in the commercial potato crisps in Kenya and also its formation as influenced by the frying temperature, potato variety and slice thickness and to determine the exposure assessment from its consumption.

1.4 Objectives

1.4.1 Broad objective

To determine the levels of acrylamide and the effects of processing on potato crisp consumed in Kenya.

1.4.2 Specific objectives

1. To assess the levels of acrylamide in potato crisps from retail outlets in Nairobi, Kenya.
2. To determine the intake levels and margin of exposures by consumption of potato crisps in Nairobi, Kenya.
3. To determine the effect of cultivar, frying temperature and slice thickness on acrylamide content of potato crisps.

1.5 Research questions

1. Are levels of acrylamide in commercial potato crisps Kenya higher than those reported in other parts of the world?
2. What are the intake levels and margin of exposures of acrylamide by the consumers who consume potato crisps in Nairobi, Kenya.
3. To what extent do cultivar, frying temperature and slice thickness influence acrylamide content of potato crisps from local cultivars?

CHAPTER TWO

LITERATURE REVIEW

2.1 Historical background

Following the leakage of acrylamide during the construction of the Hallandsås tunnel, in a control group of subjects without occupational exposures unexpected high levels of acrylamide were found in their blood (Reynolds, 2002). These findings led to the hypothesis that acrylamide might be present via dietary exposure (Tareke et al., 2000).

In April 2002 the University of Stockholm and Swedish National Food Authority announcement on the presence of acrylamide predominantly in carbohydrate rich foods sparked intensive investigations into acrylamide, involving the analysis of this compound, its occurrence, the chemical aspects, toxicology and its potential risk to the health of individuals (Tarek et al., 2002; Swedish National Food Administration, 2002). This chapter deals with review of literature on the strides made in acrylamide studies.

2.2 Processing of fried potatoes in Kenya.

2.2.1 Potato production

Potatoes (*Solanum tuberosum)* are highly rich in carbohydrate, are cheap and readily available, thus observed by consumers as an important component of a healthy, balanced diet (Henry et al., 2005). With a yearly worldwide production of about 330 million tons, the potato represents one of the world’s major staple food crops. Globally potato is the most widely grown food crop after rice, wheat and maize (MoA, 2007). In Kenya, for example, potato is the second most valuable cash and food crop after the cereal grains (MoA, 2009). Potato production in Kenya was 2,365,260 tonnes in 2011 (FAO, 2011). Intense production is, however, confined to the high altitude areas (≥2000 m above sea level) where the crop has higher production potential than maize and other cereals per unit area (MoA, 2006).

The levels of dry matter content, ratios of sugars to starch and the amount of fibre in the skin of a potato tuber changes throughout the growth period. Early harvested tubers have low dry matter content (16%) and high concentrations of glucose and fructose compared to tubers harvested at maturity or after storage. During storage sugars increases tubers begin to sprout and the tubers lose turgidity (Seal et al., 2008).

The potato is known to have high nutritive value and supplies considerable amounts of energy, minerals and vitamins (vitamin B6 and vitamin C) (Abong' et al*.,* 2009). The starch in potato tubers is converted into reducing sugars and when not all are metabolized for respiration they accumulate especially when stored under cold conditions (cold induced sweetening) or when they age through senescence sweetening. The concentration of reducing sugars determines the extent of formation of acrylamide as potatoes are generally high in the amino acid asparagine (Amrein et al., 2003).

2.2.2. Potato processing

Consumption of potato tubers are either in boiled, steamed, baked, fried or roasted forms. Nevertheless, a large percentage of potatoes are consumed as processed products, especially fried products such as French fries and potato crisps and this is of great health concern due to the high fat intake and high acrylamide content (Seal et al., 2008).The major challenge in processed potato products is obtaining optimum degree of browning and a minimal acrylamide through kinetic decoupling of different paths of maillard reaction. In Kenya, most potatoes are primarily consumed in the area of production, thus utilization is primarily in the rural areas. The consumption in urban centers has been on the increase due to the increase in the number of fast food restaurants and processing industries in most Kenyan towns especially Nairobi city (Walingo et al., 1998).

Processors involved in crisps processing prefer potato tubers that are round in shape because they easily make the required crisp diameters (Kabira and Lemaga, 2006).

2.3 Acrylamide

2.3.1 Nature and Occurrence of Acrylamide

Acrylamide also known as acrylic amide is a chemical compound of chemical formula C3H5NO. It is an industrial monomer which is very reactive and is manufactured on a large scale in Japan, the USA, and Europe through hydrolysis of acrylonitrile by nitrile hydratase. Its IUPAC name is prop-2-enamide and it is highly soluble in water (2144 g L^{-1}), ethanol, ether and chloroform. It has a low molecular weight of 71.09 g mol^{-1}. It reacts with acids, bases, oxidizing agents, iron and iron salts. Acrylamide is used in the synthesis of polyacrylamides used in water purification, soil conditioning, and proteins separation in analytical biochemistry and it is known as a component in tobacco smoke (Ahn, 2002; Grives et al., 2002). Poly-acrylamide has major application as a water soluble thickener which includes waste water treatment, gel electrophoresis, paper making, ore processing, and the manufacture of permanent press fabrics (Reynolds, 1959).

In foods acrylamide has been found to be formed in starchy foods heated at higher temperatures either through frying or baking. The maillard reaction between the amino acid asparagine and reducing sugars or reactive carbonyls at temperatures above 120°C has been suggested as possible pathway for its formation in these foods (Mottram et al., 2002; Tarek et al., 2002).

Classification of acrylamide as a Group 2A carcinogen by the International Agency for Research on Cancer (IARC) (1994) and a Category 2 carcinogen and Category 2 mutagen by the European Union (Council Directive 67/548), caused worldwide concern (WHO, 2002). In addition it has been included in the list of substances of very high concern in March 2010 by the European Chemical agency (ECHA/PR/10/05).

2.3.2 Mode of formation of acrylamide

The formation of acrylamide mainly occurs in plant based food stuffs through an irreversible combination of reducing sugars and amino acid mainly asparagine through maillard reaction. Its formation begins at 120ºC and optimal development is between 160ºC and 180ºC (Mottram et al., 2002).

Based on the studies carried out by several research groups in 2003 detailed chemical pathways to acrylamide were reported. But only Yaylayan *et al.,* (2003) and Zyzak et al. (2003) were able to show evidence for the importance of Schiff base of asparagine which corresponds to the dehydrated N-glucosyl compound. Decarboxylation of the Schiff base give acrylamide either directly or via 3-aminopropionamide (Zyzak et al., 2003). On the other hand decarboxylated amadori product which is obtained by tautomerization of the decarboxylated Schiff base may release acrylamide by a β elimination reaction (Yaylayan et al., 2003).

2.3.3 Implicated foods exposure assessment

Cereals and potatoes contain high levels of reducing sugars and the amino acid asparagine. When roasted, baked or fried they generate acrylamide with highest level of acrylamide reported in potato products (roasted and fried) and in cereal products (breads, crackers and breakfast cereals). Coffee and cocoa beans that are exposed to high temperatures during roasting have relatively high levels of acrylamide (Stadler and Scholz, 2004).

Table 1 indicates the levels of acrylamide in foods **(**Codex Alimentarius Commission in 2003).

Acrylamide exposure was estimated to be in the range of 0·3–0·8 µg/kg body weight per day based on the exposure calculations done by FAO/WHO in 2002. Exposure to acrylamide was estimated to be an average of 1 µg/kg body weight per day with the highest exposure for adults was close to 0·5 µg/kg body weight per day, with *95th* percentile values of about 1 µg/kg body weight per day. The young people in Germany aged between 15-18 years are the highest exposed since the 95th percentile was 3·4 µg/kg body weight per day. (Dybing, et al., 2005).

2.3.4 Threshold limits

WHO states that “Acrylamide belongs to the group of chemicals thought to have no reliable identifiable ‘threshold’ of effects, this means that very low concentrations will also result in very low risks, but not in zero risk (FAO/WHO 2002).

Conclusions from a consultation between FAO/WHO agreed that the "no observed adverse effect level" (NOAEL) for acrylamide neuropathy is 0.5 mg/kg body weight/day while that for fertility changes is four times higher than for peripheral neuropathy. The estimated average chronic human dietary intake is in the order of 1 µg/kg body weight/day which results in a margin between exposure and the NOAEL of 500 (FAO/WHO, 2002).

A study done by researchers at the Harvard School of Public Health and Brigham and Women's Hospital in Boston, MA on the relationship between dietary acrylamide intake and premenopausal breast cancer revealed no association between dietary acrylamide intake and breast cancer risk. The study examined 90,628 premenopausal women (Wilson, et al., 2009).

Table 2.1: Reported levels of acrylamide in fresh and processed foods.

Food / Product Group	Acrylamide levels (µg/kg)	
	Minimum	Maximum
Potato crisp	170	2510
Potatoes (raw)	<10	<50
Potatoes (boiled)	<4	<50
Potato chips/ French fries	59	1280
Corn crisps	120	220
Bakery products	24	364
Bread	<10	130
Bread (toast)	25	1430
Biscuit and crackers	18	650
Breakfast cereals	22	1400
Crisp bread	<30	1900
Noddles	11	581
Coffee (roasted)	45	374
Tea	142	567
Roasted barley grains	210	578
Chocolate products	<2	909
Nuts	28	339
Infant formulas and baby foods	<10	130
Fish and seafood products, crumbed, battered	<2	39
Poultry or game , crumbed, battered	<10	64
Beer	<6	<30

Source: Codex Alimentarius Commission, 2003

2.4 Acrylamide and Crisps

2.4.1 Acrylamide levels and effect on health

Fried potato products including crisps have, however, been reported to contain high levels of acrylamide, a carcinogenic substance of great concern not only to consumers, but also to the authorities and the food industry (Tareke et al., 2002: Viklund et al., 2008).

Acrylamide has been classified and remains a suspected human carcinogen and a neurotoxicant that calls for a concentrated effort to minimize its presence in all human diets (IARC, 1994; LoPachin., 2004; Stadler et al*.,* 2004; Viklund et al., 2010). After ingestion, acrylamide is rapidly absorbed and distributed in animals and humans throughout the whole body. It thus can be found in many body parts including the thymus, liver, heart, brain, kidneys, human placenta and breast milk thus can be transferred to the foetus or new born babies (Schettgen et al.,2004; Sorgel et al., 2002), In experimental studies done in animals acrylamide has been shown to be genotoxic attributed to its main metabolite, glycidamide (Gamboa da Costa et al., 2003; Baum et al., 2005; Doerge et al., 2005) which is mutagenic (Blasiak et al., 2004; Ghanayem et al., 2005). In pregnant women, the foetus is also exposed to acrylamide through transplacental transfer (Schettgen et al., 2004; Annola et al., 2008) and to glycidamide after maternal metabolisation of acrylamide (Annola et al., 2008). Also in the experimental animals acrylamide is responsible for tumors in several glands: thyroid, mammary, adrenal, pituitary, etc. (Johnson et al., 1986; Friedman et al., 1995). In human studies, data are more ambiguous, associations were found between dietary acrylamide and cancer in some population groups Lin et al., (2011) but not always (Burley et al., 2010; Pelucchi et al., 2011).

Acrylamide is primarily formed in fried and baked carbohydrate-rich foodstuffs such as potatoes and its formation pathway is interrelated to the Maillard reaction (reducing sugars and amino acids at elevated temperature). Potato products from Turkish markets and fast foods and restaurants, especially crisps, were found to have high levels of acrylamide (Olmaz et al., 2008). Depending on consumption, therefore, exposure to acrylamide varies depending on individuals.

Table 2 indicates the Acrylamide levels in potato chips from different countries as reported by different authors.

Table 2.2: Acrylamide levels in potato crisps from different countries

Country	**Minimum levels µg kg^{-1}**	**Maximum levels µg kg^{-1}**	**Source**
USA	462	1970	US FDA (2004)
Brazil	144	1999	Arisseto et al., (2007)
China	339	997	Chen et al., (2007)
Japan	439	1870	Ono et al., (2003)
Australia	350	618	Croft et al., (2004)
Germany	500	1000	Mattisek (2009)

In French fries, a major part of the moisture is retained in the soft core regulating the core temperature to about 100°C, though in potato crisp products moisture of the whole product is normally lowered to levels below 2·5 % resulting in core temperatures above 120°C. Therefore, acrylamide is formed throughout the whole product resulting in higher contents than in French fries despite shorter frying time. This very low moisture is necessary for the desired texture properties and storage stability of these types of potato products. Crisps processed from potatoes have tremendously gained popularity especially in major towns in Kenya (Abong’ et al., 2010). Most city dwellers consume potato crisps.

2.4.2 Exposure to acrylamide and risk assessment

Most studies on animals indicate that exposure to high levels of acrylamide leads to tumor development (Besaritinia and Pfeifer, 2003; Friedman, 2003; Rice, 2005; Spivey, 2010; Tardiff et al., 2010). There has been variation in results between acrylamide and cancer formation in humans with some indicating a relationship between acrylamide and ovarian & endometrial cancer Hogervorst et al.,(2007) though no studies has indicated a perfect relationship between breast cancer and dietary acrylamide (Larsson et al., 2009; Wilson et al., 2009 Hogervorst et al., 2007). Pedersen and others (2010) suggested a possible positive association between acrylamide and breast cancer among post-menopausal non-smokers.

Serious concerns about the safety of foods have been raised in different parts of the world due to the dietary availability of acrylamide (Halford et al., 2012). Acrylamide is a suspected human carcinogen and has been proven to be carcinogenic in animals as it interferes with the normal metabolism (Ghanayem et al., 2005; Khalil and Aziem, 2005). Studies in animals have shown carcinogenic, reproductive and genotoxic effects of acrylamide mediated partly by epoxide glycidamide, its main toxic metabolite (Blasiak et al., 2004; Baum et al., 2005; Doerge et al., 2005; Annola et al., 2008). Human studies have, however, shown varied results; some indicating positive associations of cancer with acrylamide exposure (Bongers et al., 2012) while it lacks in other studies (Lin et al., 2011; Pelucchi et al., 2011; Lipworth. et al., 2012). It therefore remains a probable human carcinogen due to limited and inconsistent human carcinogenicity evidence arising from epidemiological studies (JECFA, 2011).

Though the daily dietary acrylamide intakes vary with age and consumption, the worldwide estimation by World Health Organization is in the range of 0.3-2.0 µg/kg body weight (bw) for the total population. The intake may, however, reach 5.1 µg/kg bw when the 99 percentile is considered (JECFA, 2011). The average daily acrylamide intake by children has been reported to be 0.54 µg/kg bw/day (Heudorf et al., 2009).

When considering changes in morphology of rat nerves, the joint FAO/WHO Expert Committee on Food Additives (JECFA) established 0.2 mg/kg body weight per day to be a no-observed-adverse-effect level (NOAEL) (JECFA, 2011). In the United States, Environmental Protection Agency has set a chronic reference dose for acrylamide to be 2µg/kg/day (EPA, 2010). Margins of exposure (MOE) which is the ratio between NOAEL and dietary exposure of a given population, estimated by JECFA include 200 for the mean dietary exposure and 50 for the high dietary exposure. There is limited epidemiological data on carcinogenic effect of acrylamide, thus a dose–response relationship has been set depending on animal data with JECFA proposing lower limit on the benchmark dose for a 10% response ($BMDL_{10}$) for acrylamide to be 0.31 mg/kg body weight per day in case of induction of mammary tumors in rats and 0.18 mg/kg body weight per day for Harderian gland tumors in mice (JECFA, 2011). It therefore follows that the risk evaluation should be done by using the MOE approach which means that the ratio between the BMDL and the estimated dietary exposure is obtained.

2.4.3 Effect of Temperature, variety and thickness on acrylamide formation

It has been well documented that acrylamide can be readily formed from starch-rich foods during frying (Ono et al. 2003; Yaylayan et al. 2003). The temperature of the frying oil has been found to influence acrylamide formation with temperatures between 160°C and 180°C reported to result in the highest levels of acrylamide (Taubert et al., 2004). Chuang and others, (2008) also reported similar findings but found that the highest amount of acrylamide are formed in potatoes fried at 180°C compared to those fried at 160°C.

In French fries moisture is retained on the inner core thus temperatures during processing do not exceed 100°C. Therefore, acrylamide formation is limited to the outer dehydrated layers (Frank et al., 2005). During frying, large temperature and moisture gradients exist in these products (Gokmen et al., 2006) resulting in significantly different local reaction conditions, while in potato crisp products moisture of the whole product is normally lowered to levels below 2·5 % resulting in core temperatures above 120°C. Therefore, acrylamide is formed through the whole product resulting in higher contents than in French fries despite shorter frying times. The low moisture content is desirable in shelf life stability and textural properties. Application of lower oil temperatures requiring longer frying times for dehydration reduces final acrylamide concentrations in the product (Hasse et al., 2004).

Mathaus et al., 2004 reported that frying temperature and frying time influence acrylamide formation. Fiselier et al., (2006) and Grob, (2005) in order to maintain the desired degree of browning, recommends slight increase in frying time and reduction in the frying temperature. To avoid excessive acrylamide formation, a two-step frying process with lower temperatures in the second step should be embraced (Barry et al., 2004).

Frying at higher temperatures above 200°C can reduce final acrylamide concentrations due to intensified degradation reactions of acrylamide to glycidamide (Taubert et al., 2004 and Gokmen et al., 2006).

2.5 Review of methodologies

A great number of publications dealing with the analysis of acrylamide in foods have been published since 2002; among these are review articles on methods of analysis for the determination of acrylamide in foods (Wenzl et al., 2003; Castle et al., 2005; Zhang et al., 2005). The extraction and quantification of acrylamide has been carried out using three major methodologies that are based on chromatographic principle.

2.5.1 Liquid chromatography mass spectroscopy (LC-MS)

This method entails direct analysis of acrylamide without derivitization (Rosen et al., 2002). It utilizes the principle of aqueous extraction of acrylamide from the food matrix which is then followed by clean-up that involves single or multi stage solid phase extraction (SPE). By use of HPLC, the chromatographic separation is then done on; hydrophilic reversed phase, ion exchange column or carbon black. Major disadvantage of this method is that it can only measure acrylamide concentration levels between 20-50 µg/kg (Chakrabarti and Ungeheuer, 2002).

2.5.2 Gas chromatography mass spectroscopy with no derivitization (GC-MS with no derivitization)

This method was developed by Bridermann and Grab in 2002 for the determination of inherent acrylamide in foods and it is most laboratories use this method in acrylamide determination (Wenzl et al., 2006). When it is compared to GC-MS with derivitization it’s much faster in samples preparation and also limits the chances of handling corrosive substances. Its major disadvantage is that it needs special attention for complete extraction which requires swelling of the matrix and effective sample clean up in order to avoid artefact formation which could occur in the injection port if acrylamide precursors are contained in the sample extract (Weishaar, 2004).

2.5.3 Gas Chromatography mass spectroscopy with derivitization (GC-MS with derivitization)

This method involves aqueous extraction of acrylamide from the matrix followed by derivitization of acrylamide to 2, 3 – dibromopropionate. It involves the use of elemental bromine or potassium bromate (Nemoto et al., 2002). The extraction using this method is much faster and the analyte is easy to detect because the derivative is less polar than native acrylamide. To avoid uncontrolled partial dehybromination in the injection port, triethyl amine in a variant of this method is added, thus its major disadvantage.

2.6 Research gaps

The contribution of commercial potato crisps in terms of acrylamide, intake levels and margin of exposures are supported by limited data in Kenya. There is also insufficient data on the effect of processing on acrylamide levels. This study was designed to fill this knowledge gaps.

CHAPTER THREE

Levels of Acrylamide in Commercial Potato Crisps Sold in Nairobi, Kenya

Abstract

Acrylamide has been found to be genotoxic and a neurotoxicant and its classification as a suspected human carcinogen calls for a concerted effort to minimize its presence in human diet. It is mainly formed in fried and baked carbohydrate rich foodstuffs such as potato chips and crisps through maillard reaction at elevated temperatures. This study was designed to determine the levels of acrylamide in commercial potato crisps that are sold in Nairobi, Kenya.

Different brands of potato crisps were purchased from retail outlets while unbranded (street) samples were purchased from kiosks in five districts of the Nairobi County. The samples purchased were a total of 35 branded samples and fifteen unbranded samples. The parameters analysed were moisture, colour and acrylamide content. The moisture content of the crisps ranged from 0.39% to 7.97%. There was a significant ($P \leq 0.05$) difference among the crisps samples in color parameters. Out of the forty three products, most of the samples were light colored with lightness (L*) parameters greater than 50 apart from only two samples. Most samples tended towards green as shown by the negative values of redness parameter (a*) indicating that there was less or no excess browning of the products during frying. All the samples tended towards yellow as indicated by positive values of yellowness parameter (b*).

Acrylamide levels significantly ($P \leq 0.05$) differed between the traded crisps brands ranging from non-detectable levels to 8666 μg kg^{-1} in the branded samples while in the unbranded samples it ranged from 5666 μg kg^{-1} in Kiosk 7 to 9499 μg kg^{-1} in Kiosk 6. There was a significant differences ($P<0.05$) in acrylamide levels between the branded and the unbranded (street) potato crisps. Most of the flavoured brands were non-detectable. It therefore indicates that depending on the brand of crisps and amounts consumed by an individual, levels of acrylamide intake and exposure in Nairobi will differ greatly.

Key words: Acrylamide, potato crisps, carcinogen, processing.

3.0 Introduction

Potatoes (*Solanum tuberosum)* are high in carbohydrate and vitamin C, and are perceived by consumers as an important component of a healthy balanced diet. With a yearly worldwide production of approximately 330 million tons, the potato represents one of the world's major staple food crops. Globally, potato is the fourth most widely grown food crop after rice, wheat and maize (MoA, 2007). Potato comes second as the most valuable cash and food crop after the cereal grains in Kenya according to the Ministry of Agriculture (MoA, 2009). Potato crisps and French fries are the most consumed potato products (Kulkarni et al., 1994; Knol et al., 2009). According to the East African Standards (2010) and Salvador and others (2008a), a potato crisp is defined as a fragile but firm slice of potato which has been cooked by deep frying in vegetable oil and to which edible salt (powder or brine) or permitted food grade spices, colour and flavour may have been added.

Acrylamide has been classified as a Group 2A carcinogen by the International Agency for Research on Cancer (IARC, 1994) and remains a suspected human carcinogen and a neurotoxicant that calls for a concerted effort to minimize its presence in all human diets (LoPachin, 2004; Stadler et al., 2004; Viklund et al., 2010). Acrylamide is mainly formed in fried and baked carbohydrate-rich foodstuffs such as potatoes as its formation pathway is linked with the reaction involving sugars and free amino acids at elevated temperature.

The estimated exposure to acrylamide has been shown to depend on dietary habits of consumers in different countries (Dybing et al., 2005). Consumption patterns vary among people of different ages, and younger people for instance, tend to eat more snacks than older people (Konings et al., 2003), which may result in a higher intake of acrylamide among the younger generation and hence higher potential risk of developing cancer related disorders. The consumption pattern is similar in Kenya and has been on the increase (Abong' et al., 2010). Crisps processed from potatoes have tremendously gained popularity especially in major towns in Kenya (Abong' et al., 2010). Most city dwellers consume potato crisps as snack between meals and during festivities.

The acrylamide content in fried potato products can be related to color (Pedreschi et al., 2005) and hence the need to monitor color parameters of acrylamide prone products such as potato crisps. Color is also an important quality parameter that determines the acceptance of fried potato products.

Since there is limited data on acrylamide content in potato crisps in the Kenyan market, this study aimed at evaluating the levels of acrylamide in commercial crisps in the Kenyan market and determining the relationship between color and acrylamide formation. In addition, the comparison of the acrylamide content in branded and in the unbranded potato crisps was done. The acquired information was useful in determining the level of exposure to acrylamide potato crisps consumers.

3.1Materials and methods

3.1.1 Survey of acrylamide contents in potato crisps

3.1.1.1 Study site

The study was carried out in Nairobi County, Kenya. Nairobi is the capital city of Kenya and has eight constituencies: Makadara, Embakasi, Starehe, Langata, Kasarani, Westlands, Kamukunji and Dagorreti. The main administrative divisions are Kibera, Makadara, Westlands, Central, Dagorretti, Embakasi, Kasarani and Pumwani. According to the 2009 census the population is estimated to be 3,138,295 with a total area of 696 km^2. It is a cosmopolitan and multicultural city. Half of the population lives in slums, while most of the up market suburbs are situated to the west and north central of Nairobi, Lower middle and upper middle income located in the north central areas while low and lower income estates in eastern Nairobi. Most of the food production industries are located in the industrial area.

3.1.1.2 Study type and design

This study was carried out in Nairobi city and its environs. Nairobi was purposively selected due to the large number of factories and outlets that process crisps (Abong et al., 2009). The study was a cross-sectional survey and applying quantitative data collection methods through laboratory analysis.

3.1.1.3 Sample size determination

Sampling frame was obtained in which number of retail outlets dealing in potato crisps was established. The total number obtained from the listings was found to be 140 supermarkets (www.best-kenya-safaris.com/kenya) in Nairobi, from this, 30 outlets was randomly selected. Also from the five districts selected a total of thirty samples were purchased from the roadside kiosks.

3.1.1.4 Sampling procedure

This study employed purposive sampling and simple random sampling techniques. The sampling type employed was purposive sampling. Nairobi comprises nine districts. Five districts was purposively selected which include: Starehe, Embakasi, Westlands, Kasarani and Langata. 30 supermarkets were purposively selected which stock various brands of crisps. From Starehe 10 supermarkets were selected purposively while five from each of the remaining four districts. 30 samples was purposively purchased from Starehe district as it encompasses the central business district (CBD) thus accessibility by a large number of the population. The remaining 40 samples were purchased 10 from each district. A total of 35 samples of branded crisps each weighing 50g were randomly picked from the supermarkets in duplicates. Three Vendors from each of the five districts were purposively selected. For the unbranded crisps six samples were purchased randomly from each of the five regions in duplicates. Data was collected on type of outlet and their locations, brands, moisture, color and acrylamide contents.

Figure 1 gives a schematic representation of the sampling procedure.

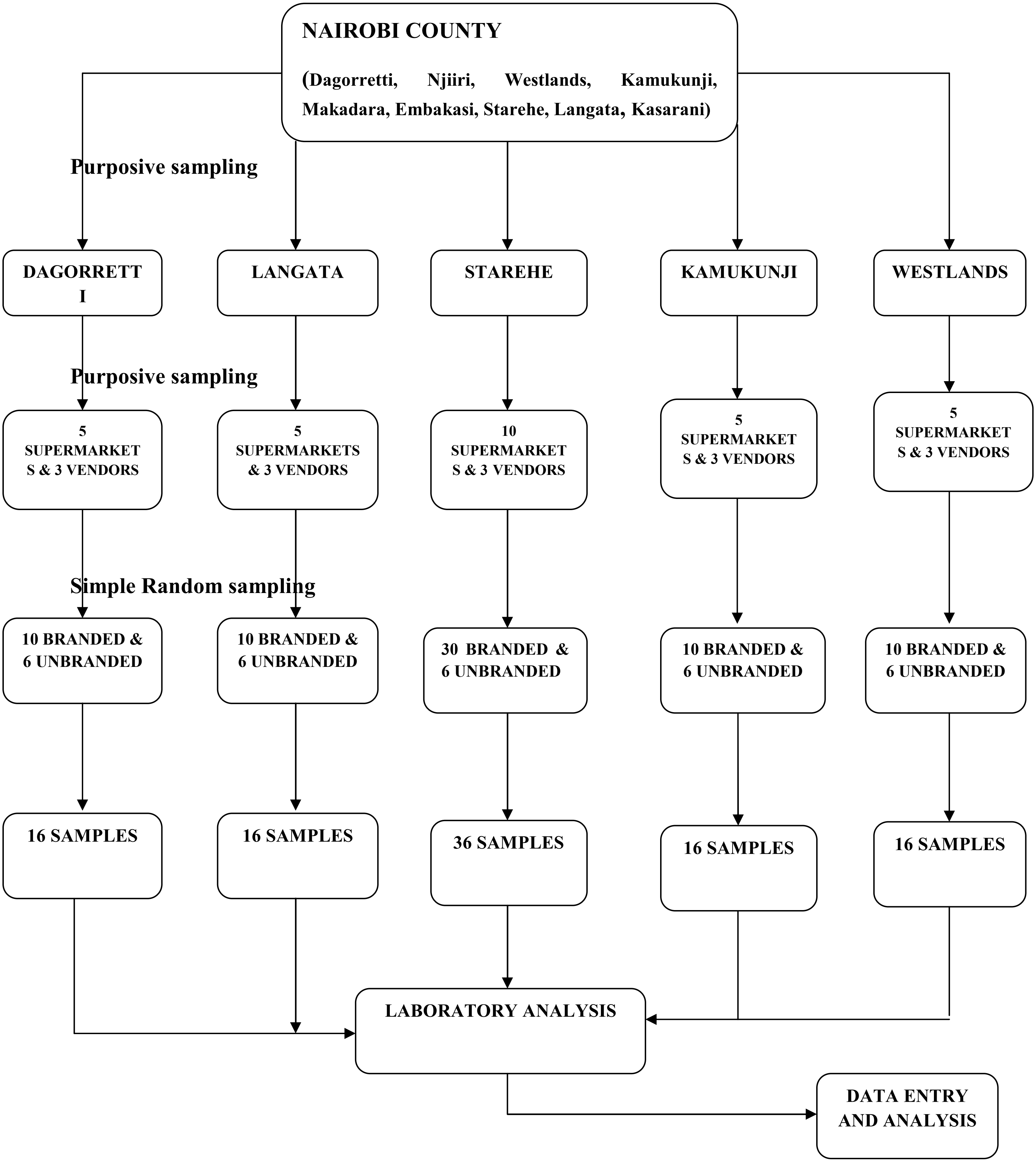

Figure 1: Schematic presentation of the sampling Procedure.

3.1.2 Techniques of data collection

This involved quantitative data collection through laboratory analysis of the commercial potato crisps purchased.

3.2 Analytical methods.

3.2.1 Determination of moisture content

The moisture content of the potato crisps was determined by oven drying according to AOAC (1980). The oven used was of model DS0-500D made in Israel.

3.2.2 Determination of color of crisps

The colours of the potato crisps were evaluated using a color spectrophotometer as described by Abong' et al. (2011). The potato crisps color was measured with a color spectrophotometer NF 333 (Nippon Denshoku, Japan) using the CIE Lab L*, a* and b* color scale. The 'L*' value which is the lightness parameter indicating degree of lightness of the sample varies from 0=black to 100=white. The 'a*' is the chromatic redness parameter whose value means tending to red color when positive (+) and green color when negative (–). The 'b*' is yellowness chromatic parameter corresponding to yellow color when it is positive (+) and blue color when it is negative (–). Each sample was measured six times.

3.2.3 Determination of acrylamide

Levels of acrylamide were determined as described by the United States Food and Drug Administration (FDA) method *Detection and Quantitation of Acrylamide in Foods* (2002) using a Gas chromatography and Flame ionizable detector (G-14B,Shimadzu, Japan).

Acrylamide extraction

The samples were crushed and 1g was combined with 10 ml of 0.1% formic acid solution and the mixture was then mixed on a test tube shaker (KS250, Japan) for 5 minutes at 300 rpm. The samples were then centrifuged at 3000 rpm for 10 min to ensure easier removal of the oily top layer and ensure further solubility of the acrylamide compound. The supernatant was extracted then filtered using a filter paper (Wattman paper no. 47) before being passed through a 0.45µm nylon syringe filter to remove any suspended particles and then stored in a refrigerator awaiting the cleanup and analysis stage.

Clean up stage

Clean up involved passing the filtered sample through a solid phase extractor tube (Carboprep tm 200 SPE tube, 6ml, 500mg) which was first activated by passing 2 ml of acetone solvent and then 2 ml of 0.1% formic acid. The filtered sample solution was then passed through the tube and allowed to flow under gravity. Water (1 ml) was then fast passed through the tube. The SPE tube was then dried in a vacuum for one minute after which 2 ml of analytical grade acetone flowing through gravity was passed for elution. 1 microliter of the elute was injected into the column and the chromatogram interpreted. The elute was stored immediately in the refrigerator ready for the GC-FID analysis.

Gas chromatography conditions

The column used was a supelcowax capillary column since it gave good results during determination of the suitable condition for analysis using this column. The injection temperature was maintained at 260°C while nitrogen, the carrier gas, was supplied at 100 bars pressure. The linear velocity of the carrier gas was maintained at 62cm/sec at 100 °C, while the oven temperature was set at 100 °C then held for 0.5 min before it was allowed to increase at a rate of 15 °C/min to attain a final temperature of 200 °C . The limit of detection for the GC-FID was found to be 4.5ppm while the Limit of quantification was 45ppm.

3.3 Data analysis

Both descriptive and inferential statistical tests including analysis of variance (ANOVA) and least significant difference test (LSD) for the variables were carried out using the Statistical Analysis System (SAS) version 9.1.3. Pearson correlation analysis and multiple regression analysis were also performed to determine relationships between acrylamide and moisture and acrylamide and colour parameters. Where the differences of $p \leq 0.05$ existed, the samples were considered to be significant.

3.4 Results and Discussion

3.4.1 Results

The color parameters of different brands of crisps sold in Nairobi, Kenya are given in Tables 3.1.

Table 3.1: Color of potato crisps sold in Nairobi, Kenya.

Brand	L*	a*	b*
BC1	67.2±3.6cde	7.3±1.2c	35.3±2.3a
BC1 flavoured	67.7±4.7cde	-3.2±1.9stu	30.0±5.9bcdefgh
BC2	62.2±7.7efghijk	-1.7±1.2nopqrst	24.8±3.3mnopqrs
BC3 flavoured	57.4±6.6ijklmop	12.4±3.8a	30.9±2.8bcdef
BC3	59.9±7.8hijklmn	0.9±2.8hijklm	30.6±5.2bcdefg
BC4	67.1±7.2cdef	-3.2±1.4rstu	17.4±2.5x
BC5 flavoured	53.7±9.2op	10.1±5.2ab	25.7±lmnopqr
BC5	62.3±3.5efghijk	-1.69±1.1nopqrst	29.±3.9defghij
Vendor 1	54.7±6.9nop	2.5±3.1efghij	25.6±4.2lmnopqr
Vendor 2	59.6±4.6ijklmno	2.1±3.9efghijk	26.2±5.5klmnop
Vendor 3	52.3±2.9pq	0.9±1.4hijklm	25.7±3.1lmnopqr
Vendor 4	63.2±4.7efghij	7.0±5.7cd	33.3±3.6ab
Vendor 5	59.9±9.1hijklmn	4.4±3.6def	31.5±3.9abcd
Vendor 6	59.2±3.3ijklmno	3.3±1.6efgh	32.3±3.8abc
BC6	67.4±4.0cde	0.7±1.5hijklm	25.4±4.4mnopqrs
BC7	58.5±5.9hijklmno	1.9±1.4ghijk	21.4±9.4tuvwx
BC8	54.0±6.5nop	0.6±2.1ijklmn	21.6±4.4qrstuv
BC9	66.6±4.4cdefg	-2.6±0.5qrstu	28.2±1.1ghijklmn
BC10	63.9±5.8defgh	-3.1±0.7rstu	24.2±4.6nopqrst
BC11	70.1±3.2bc	-3.7±0.6ut	28.2±2.9fghijklm
BC12	77.±2.2a	-3.0±0.3rstu	25.1±1.7mnopqrs
BC13	69.7±4.5cd	0.3jklmno	26.0±jklmnop
BC14	61.8±6.7efghijk	-0.6±3.5mnopqr	31.2±2.0abcdef
BC15	59.8±5.4hijklmn	-2.4±0.53qrstu	24.3±3.3nopqrst
BC15 flavoured	54.2±3.0nop	4.6±1.7de	23.1±3.8opqrstu
BC16	75.9±2.4ab	-2.6±0.5qrstu	21.5±1.4rstuvw
BC17 flavoured	53.7±3.0op	10.7±2.7ab	29.6±3.8defghij
BC17	62.3±5.2efghijk	-0.5±0.5mnopqr	30±3.3defghi
BC18	63.0±6.1efghij	0.2±2.4jklmnop	27.2±2.4hijklmno
BC19	57.3±6.3ijklmnop	0.5±0.5ijklmn	19.7±2.9vwx
Vendor 7	56.6±5.9lkmnop	0.8±4.7hijklm	26.4±4.0jklmnop

[1] Values are means of six determinations ± standard deviation.

[2] The values with similar letters in the same column are not significantly different at 5% level of significance.

Table 3.1 Continued.

Brand	L*	a*	b*
Vendor 8	56.5±6.0klmnop	-1.1±2.1nopqrst	22.3±3.1pqrstuv
Vendor 9	55.6±5.5lmnop	0.2±2.2jklmnop	24.0±5.5nopqrst
BC20	70.7±4.4bc	-1.5±0.5nopqrst	28.2±1.5ghijklmn
BC21	47.1±3.8q	4.0±1.4efg	20.4±1.3uvwxt
BC22	59.9±4.4hijklmn	-1.7±2.2nopqrst	25.8±2.5klmnopq
BC23	59.6±6.6hijklmno	0.9±1.7hijklm	20.2±4.0uvwx
BC24	63.2±3.1efghi	-1.7±0.8nopqrst	24.4±1.6nopqrst
BC25	56.9±6.7klmnop	1.4±4.4ghijkl	31.1±3.3bcdef
BC26	72.1±3.9abc	-4.7±0.2u	18.3±1.1wx
BC27	64.0±2.3defgh	0.8±1.3hijklm	21.7±2.8qrstuv
BC28	59.1±7.5ijklmno	-2.1±1.7opqrstu	27.3±2.3hijklmn
BC29	62.2±3.0efghijk	-1.7±0.5nopqrst	28.6±1.5hijklm
Vendor 10	55.1±3.2mnop	1.9±3.6efghijk	26.3±5.6jklmnop
Vendor 11	56.4±6.0lmnop	2.3±2.5efghij	27.2±4.3hijklmno
Vendor 12	51.5±4.3qp	2.4±1.6efghij	25.5±2.3mnopqrs
BC30	55.3l±7.0mnop	0.6±1.1hijklm	26.9±2.0ijklmno
BC31	66.6±6.1cdefg	-1.5±3.8nopqrst	26.6±4.9jklmnop
BC31 flavoured	54.2±6.4nop	8.3±2.2bc	19.1±2.6vwx
BC32	67.7±5.8cde	-2.1±2.6opqrstu	30.6±6.2bcdefg
BC33	60.9±5.0ghijklm	-3.7±1.2ut	20.2±1.9uvwx
BC33 flavoured	56.4±2.1klmnop	4.0±1.8efg	23.1±2.6opqrstu
Vendor 13	54.6±2.6nop	-0.1±0.8klmnopq	31.8±3.1abc
Vendor 14	60.8±4.7ghijklm	-1.2±1.2nopqrst	31.4±5.0abcde
Vendor 15	61.2±3.5fghijkl	-2.2±1.6opqrstu	31.9±4.5abc
BC34 flavoured	59.6±4.0ijklmno	4.2±2.3ef	25.3±2.3mnopqrs
BC34	57.2±4.3jklmnop	-2.4±1.1opqrstu	24.6±3.8nopqrst
BC35	55.0±4.8 mnop	2.8±4.1efgh	29.1±3.7defghijk

[1] Values are means of six determinations± standard deviation.

[2] Values with the same letters in the same column are not significantly different at 5% level of significance.

BC: Branded Crisps

All the brands purchased were light colored with L*>50 apart from that of BC21; the lightest brand was BC12 with a lightness level of 77.2. The least yellow was BC4 while the highest value of yellowness parameter was the unbranded sample obtained from BC1 (b*=35.3). Most of the samples tended towards green as the redness parameter veered towards the negative indicating low degree of browning. Most flavoured samples had high values of the redness parameter compared to the other branded samples. The positive value of the yellowness parameter b* is an indication that all the potato crisps in the Kenyan market tend towards yellow.

The percentage moisture content of different brands of potato crisps sold in Nairobi is given in Table 3.2.

Table 3.2: Moisture content of potato crisps sold in Nairobi, Kenya.

Brand	%Moisture content	Brand	% Moisture content
BC1	3.10±0.0ijklm	BC19	4.07±0.18defghij
BC1flavoured	4.034±0.35defgh	Vendor 7	2.92±0.25jklmn
BC2	3.03±0.67jklmno	Vendor 8	2.96±0.09ijklmn
BC3 flavoured	4.87±0.40cdefg	Vendor 9	3.18±0.09hijklm
BC3	5.27±0.02bcd	BC20	1.0±0.23qrs
BC4	3.89±0.10efghijk	BC21	7.05±1.3a
BC5	1.64±0.37opqrs	BC22	2.43±0.6mnop
BC5 flavoured	2.91±0.73jklmn	BC23	3.78±0.32ghijkl
Vendor 1	4.88±0.19cdef	BC24	2.48±0.53lmnop
Vendor 2	5.16±0.57bcde	BC25	4.13±0.01defghij
Vendor 3	6.09±0.05abc	BC26	0.88±0.0rs
Vendor 4	5.98±0.70abc	BC27	3.87±0.16efghijk
Vendor 5	4.97±0.37cdef	BC28	4.18±0.31defghi
Vendor 6	4.99±0.10cde	BC29	1.58±0.31opqrs
BC6	2.98±0.11ijklmn	Vendor 10	2.93±0.70jklmn
BC7	3.29±0.01hijklm	Vendor 11	3.16±1.12hijklm
BC8	5.37±0.11bcd	Vendor 12	3.3±0.08hijklm
BC9	3.85±0.24efghijk	BC30	3.97±0.18efghijk
BC10	3.62±0.03ghijkl	BC31	0.99±0.155qrs
BC11	5.19±0.01bcde	BC31 flavoured	3.1±1.2ijklm
BC12	1.71±0.18nopqrs	BC32	4.13±0.01defghij
BC13	4.73±0.21cdef	BC33	1.62±0.20opqrs
BC14	4.29±0.35defghi	BC33 flavoured	3.37±0.31hijklm
BC15 flavoured	1.07±0.21qrs	Vendor 13	5.05±0.06bcde
BC15	0.39±0.10s	Vendor 14	4.50±0.05defgh
BC16	0.67±0.07rs	Vendor 15	5.24±0.04bcd
BC17 flavoured	1.22±0.07pqrs	BC34 flavoured	4.48±0.05defgh
BC 17	0.97±0.21qrs	BC34	2.04±0.03mnopq
BC18	4.47±0.18defgh	BC35	4.26±1.0hijklm

[1] Values are means of two determinants ± standard deviations.

[2] Values with the same letters in the column are not significantly different $p<0.05$.

The percentage moisture content in branded samples varied significantly among all the samples $p<0.05$ ranging from 0.39 in crisps 16 to 7.05% in BC21. In the unbranded samples the moisture content ranged from 2.93% from vendor 5 to 6.09% from Vendor 3. Table 3.3 below indicates the levels of acrylamide in potato crisps from Nairobi.

Table 3.3: Acrylamide levels of potato crisps sold in Nairobi.

Brand	**Acrylamide µg/kg**	**Brand**	**Acrylamide µg/kg**
BC1	6934.4±160defghijk	Vendor 8	6156±1441hijklm
BC1flavoured	ND	Vendor 9	7797±322bcdefgh
BC2	7753±489bcdefh	BC18	8667±377abcd
BC3	3939±321opqr	BC19	7883±559abcdefgh
BC3 flavoured	ND	BC20	1986±89st
BC 4	2776±208qrst	BC21	8417±33abcdefg
Vendor 1	8556.8±1986abcdef	BC22	3834±94nopqrs
Vendor 2	8959.1±311abc	BC23	6605±518fghijkl
Vendor 3	8711.6±298abcd	BC24	3192±205nopqrs
BC5	2895±85qrst	BC25	7739±543bcdegh
BC5 Flavoured	ND	BC26	ND
Vendor 4	9449.3±199ab	BC27	4876±649lmnop
Vendor 5	8739.1±108abcd	BC28	2727±972qrst
Vendor 6	9728.1±276a	BC29	3903±534nopqr
BC6	5311±391klmn	Vendor 10	6678±1489fghijkl
BC7	7317.6±1503cdefgi	Vendor 11	7811±737bcdefgh
BC8	3855±9nopqrs	Vendor 12	8499±448abcdef
BC9	4342±52mnopq	Vendor 30	7497±690bcdefgh
BC10	3076±701opqrst	BC31	4995±470lmno
BC11	6721±823fghijkl	BC31 Flavoured	ND
BC12	2380±241rst	BC32	4267±1313nopq
BC13	8608.8±267abcde	BC33	ND
BC14	8010.5±477abcdefg	BC33 flavoured	2558±320qrst
BC15	ND	Vendor 13	5225±922klmn
BC15 flavoured	ND	Vendor 14	7785±221bcdefgh
BC16	1506±382st	Vendor 15	6746±867defghijk
BC17	5606±444klmn	BC34	5312±344klmn
BC17 flavoured	5151±269klmn	BC34 flavoured	ND
Vendor 7	5666±286hijklm	BC35	2138±78rst

[1] Values are means of two determinants ± standard deviations.

[2] Values with the same letters in the column are not significantly different $p<0.05$.

The acrylamide levels significantly differed among the brands and place of purchase ranging from non-detectable levels to 8666 μg kg−1 in BC18 for the retail samples while in the unbranded (street) samples the levels ranged from 5666 μg kg−1 in Vendor 7 to 9499 μg kg−1 in Vendor 6. The levels were significantly higher in street samples compared to retail samples. All the flavoured samples, the acrylamide levels were non-detectable.

The correlation between moisture content, acrylamide content and the colour parameters is shown in Table 3.4.

Table 3.4: Correlation between acrylamide content, moisture content and colour parameters for potato crisps commercially available in Nairobi, Kenya

Parameters	Acrylamide	Moisture
Acrylamide	1.000	0.369**
Moisture	0.369**	1.000
L*	-0.089a	-0.313*
a*	0.189a	0.163a
b*	0.334*	0.048a

(n=86); a not significantly different at $p>0.05$

*Significantly different at $p<0.05$

** Significantly different at $p<0.001$

There is a weak positive relationship between the acrylamide contents and the moisture content where r=0.36942.

There exists a very weak positive relationship between the degree of redness and the acrylamide content r=0.1889. This finding agrees with what has been reported by other authors (Pedreschi *et al.,* 2005). No relationship between the degree of lightness and the acrylamide content was, however found to exist.

3.4.2 Discussion

Excessive browning of the food products increases at higher temperatures, the acrylamide compound is broken down to glycidamide thus its concentration reduces hence the weak positive relationship between the degree of redness and the acrylamide content. The reducing sugar content of potatoes can be considered the limiting factor for the potential of acrylamide formation. This is because the level of asparagine in raw potatoes varies only within a narrow range, whereas the reducing sugar content can vary widely depending on variety, maturity, and storage among other growing and processing conditions. State of maturity and storage conditions influence sugars levels and hence directly affect the amount of acrylamide present in fried potato products (Olsson et al., 2004; De Wilde et al., 2005; Abong' et al., 2009). The high concentration of the acrylamide in the commercial Kenyan potato crisps can be related to high reducing sugars in the raw tubers and high frying temperatures. BC23 not only had the lowest value of L* but also the highest a* value, an indication that the crisps were produced from tubers with reducing sugars greater than 0.25% which is required for processing. Colour and taste development in potato crisps has been known to be influenced by the Maillard reaction between amino acids and reducing sugars (Pedreschi et al., 2005; Santis et al., 2007; Hassananpanan et al., 2011). Significant differences ($P<0.05$) were noted between different brands in all the color parameters.

Percentage moisture content in the samples ranged from 0.39 to 7.53 which were slightly higher than those reported by Abong et al. (2009). This is due to the increase in the number of brands available in the market as they have increased from 25 reported to 38 by Abong et al. (2011). The EAS (2010) recommends maximum crisps moisture of 5%. In this study, 99% of the branded samples had the recommended moisture content. Thus the products are shelf life stable.

However, the most of the samples (80 %) purchased from the kiosks, had moisture content higher than the maximum recommended levels. These products may therefore not be shelf-stable as moisture influences shelf-life of products High moisture content can be related to the type of packaging such as polythene bags that have been tampered with thus allowing water percolation into the crisps and also inadequate drying before frying.

Pre-treatments before frying decreases the acrylamide content as can be seen in the branded samples of BC33 which are seasoned before frying; BC18 have only about 42% potato content, the rest comprising wheat, starch and flours (potato, corn, and rice) mixed with vegetable oils and an emulsifier..

In potato crisps, moisture of the whole product is normally lowered to levels below 2·5 % which is important for the desired textural properties and prolonged shelf life. When the moisture content is lowered below these levels, the core temperature rise above 120°C, acrylamide is thereby formed throughout the product resulting in higher contents (Taubert et al., 2004).

The formation of acrylamide mainly occurs in plant based food stuffs through an irreversible combination of reducing sugars and amino acid mainly asparagine the maillard reaction. Its acrylamide formation begins at 120ºC and optimal development occurs between 160ºC and 180ºC (Mottram et al., 2002). The use of low frying temperature (under 160°C) reduces the concentration of acrylamide produced but this negatively affect the quality characteristics of the product like texture, color and oil content and consequently the acceptability of the product by the consumer may be reduced. Higher values of acrylamide are an indication of higher level of exposure to this potential carcinogen, neurotoxic and genotoxic substance. The average dietary exposure to acrylamide for children was found to be about two times higher than that for adults in European populations (Arribas-Lorenzo and Morales, 2009). This could be similar to that in

Kenya since young people tend to consume higher amount of this snack (Abong., et al. 2010) due to their lower body weight and high caloric demand.
Levels of acrylamide in the flavoured potato crisps were non-detectable and thus need to do more studies to determine the effect of flavouring compounds on acrylamide detection.

Taubert et al. (2004) found that acrylamide levels increase with greater temperature and cooking time in potatoes with low surface-to-volume ratios (SVR), whereas for potatoes with high surface-to-volume ratios acrylamide levels peak and then decline with further heating. Variation may be a function of the availability of substrate since in low SVR potatoes, there is a larger, steady supply of sugars and asparagine, but in high SVR potatoes, the supply is more quickly depleted.

Maximum limits for acrylamide in food have, however, not been established. The WHO guideline for acrylamide in drinking water is 0.5µg/kg but similar information is lacking for foodstuffs (WHO, 2002). According to these high levels of acrylamide, crisps consumers in Kenya may be exposed to risks associated with acrylamide.

3.5 Conclusions and Recommendations

Acrylamide content of unbranded potato crisps are higher than those of the branded potato crisps. There exists a very weak positive relationship between the degree of redness and the acrylamide, however, no relationship between the degree of lightness and the acrylamide content exists. Ministry of Public Health and Kenya Bureau of Standards should take the lead in ensuring that the code of conduct to reduce acrylamide is adhered to. Regular monitoring of fried products and risk analysis should be carried out in the Kenyan markets and measures taken to reduce the levels of acrylamide in the commercial potato crisps.

CHAPTER FOUR

Dietary Acrylamide Exposure of Kenyan Population: Case Study of Potato Crisps Consumers in Nairobi

Abstract

Acrylamide is a heat induced possible human carcinogenic chemical resulting from irreversible combination between reducing sugars and amino acid asparagine especially when foodstuffs are cooked at temperatures of 120°C and above. Since plant foodstuffs such as potato have high asparagine, acrylamide tends to be high in fried potato products such as French fries and potato crisps. Potato crisps for instance provide between-the-meal snacks to many consumers all over the world including Kenya. This study was intended to assess the exposure to acrylamide by consumption of potato crisps in Nairobi, Kenya. Potato crisps consumption survey was carried out among crisps consumers. The data were collected from the consumers from the retail outlets where they bought the crisps. Consumption data were combined with contamination data arising from analysis of crisps at retail outlets and street processors in the same region and dietary acrylamide exposure was calculated using probabilistic approach. The mean acrylamide intake was 1.57µg/kg bw/day while the 95th (P95) percentile was 5.1µg/kg bw/day with Margins of Exposures (MOE) being respectively 197 and 61. The acrylamide intake was significantly higher in street processed crisps (non-branded) with a mean value of 2.26 µg/kg bw/day and 95th percentile of 6.54 µg/kg bw/day, and MOE being respectively 137 and 47. The extremely lower MOEs indicate higher exposure to acrylamide by the consumers mainly due to higher contamination levels. Important steps are therefore required to mitigate the high exposure by reducing level of acrylamide contamination and decreasing consumption.

Key words: Exposure, acrylamide, carcinogen, potato crisps

4.0 Introduction

All over the world, there has been a general trend of increased consumption of fried potato products (Peksa et al., 2006) with potato crisps and French fries being the most important potato products that are industrially produced and consumed by a larger proportion of urban dwellers, Kenya being included (Abong' et al., 2010). The increased consumption is mainly linked to the young generation who find the products convenient with unique appealing sensory properties (Chiou et al., 2012). The fried potato products have, however, been shown to contain life threatening by-products such as acrylamide depending on level of contamination and consumption.

Acrylamide was first discovered in high carbohydrate foods mainly in potato and cereal products by Swedish researchers in 2002 (Tareke et al., 2002). After these findings, several studies including chemistry, toxicology, exposure and possible risks have been carried out in different parts of the world (Hogervorst.et al., 2008). It is widely agreed that acrylamide is a monomer mainly formed through acrylamide reaction involving free asparagine and carbohydrates, mainly reducing sugars. Asparagine, the backbone of the acrylamide molecule, is necessary in the reaction while reducing sugars are essential co-reactants especially in the formation of the N-glycoside intermediates (Mottram et al., 2002). Acrylamide is a ubiquitous chemical in the diet, meaning that it is almost impossible to totally eliminate its exposure in fried or baked foods. The extent of acrylamide formation is, however, influenced by temperature; formation usually increases above 120°C depending on the food matrix (Becalski et al., 2003; Stadler and Scholz, 2004). Together with French fries, potato crisps have been shown to have high levels of acrylamide in most recorded research reports (Fan and Mastovska, 2006; Pedreschi et al., 2010b; Sirot et al., 2012). The high level of contamination has been attributed to high level of free asparagine in potatoes compared to other foods which undergo similar processing conditions (Zyzak et al., 2003). Cereal products such as bread, breakfast cereals, biscuits cakes and coffee have also been implicated as major sources of acrylamide. Variation in acrylamide can be quite high in different foods. For instance, in Europe where adequate monitoring was undertaken for three years, the mean levels of acrylamide was found to range from as low as 37 µg/kg for soft drinks and as high as 1504 µg/kg for substitute coffee, while the highest 95th percentile and maximum levels were recorded in substitute coffee at 3976 and potato crisps at 4804 µg/kg, respectively (EFSA, 2011).

The presence of acrylamide in food products has raised serious food safety concerns all over the world (Halford et al., 2012). Due to adverse effects indicated on normal animal metabolism, acrylamide remains a suspect human carcinogen and a neurotoxicant (Ghanayem et al., 2005; Khalil and Aziem, 2005). Studies in animals have shown carcinogenic, reproductive and genotoxic effects of acrylamide mediated partly by epoxide glycidamide, its main toxic metabolite (Blasiak et al., 2004; Baum et al., 2005; Doerge et al., 2005; Annola et al., 2008). Animal studies have also indicated increased incidences of tumor in different glands such as pituitary, adrenal and mammary glands following exposure to acrylamide (Friedman et al., 1995). Human studies have, however, shown varied results; some indicating positive associations of cancer with acrylamide exposure (Bongers et al., 2012) while it lacks in other studies (Lin et al., 2011; Pelucchi et al., 2011; Lipworth. et al., 2012). It therefore remains a probable human carcinogen due to limited and inconsistent human carcinogenicity evidence arising from epidemiological studies (IARC, 1994; JECFA, 2011). Acrylamide remains in the proposition 65 list of chemicals known by the state (USA) to cause cancer or reproductive toxicity since 1990 (OEHHA, 2012).

Though the daily dietary acrylamide intakes vary with age and consumption, the worldwide estimation by World Health Organization is in the range of 0.3-2.0 µg/kg body weight (BW) for the total population. The intake may, however, reach 5.1µg/kg bw when the 99 percentile is considered (JECFA, 2011). The average daily acrylamide intake by children has been reported to be 0.54 µg/kg bw/day (Heudorf et al., 2009). In a Polish study, however, exposure of infants (6–12 months) was found to be quite higher than general population being estimated at the minimum to range from 0.41 to 0.62 µg /kg bw/day, on the average level it ranged from 2.10 to 4.32 µg /kg bw/day while for the worst case scenario the exposure ranged from 7.47 to 12.35 µg /kg bw/day (Mojska et al., 2012). In Europe the mean acrylamide exposure has been estimated to range between 0.31 and 1.1 µg/kg bw per day for adults over 18 years, and between 0.43 and 1.4 µg/kg bw per day for adolescents (11-17 years). The estimate is, however, high for children ranging between 0.70 and 2.05 µg/kg bw per day for children (3-10 years) and between 1.2 and 2.4 µg/kg bw per day for toddlers (1-3 years) with major contributors to exposure for adults being fried potatoes, coffee, and soft bread while for adolescents and children they were French fries, soft bread, potato crisps, and biscuits (EFSA, 2011).

The joint FAO/WHO Expert Committee on Food Additives (JECFA) established 0.2 mg/kg body weight per day to be a no-observed-adverse-effect level (NOAEL) when considering changes in morphology of rat nerves (JECFA, 2011). In the United States, Environmental Protection Agency has set a chronic reference dose for acrylamide to be 2µg/kg/day (EPA, 2010). Margins of exposure (MOE) estimated by JECFA include 200 for the mean dietary exposure and 50 for the high dietary exposure. The MOE corresponds to the ratio between the NOAEL and the dietary exposure of a given population. Due to insufficient epidemiological data on carcinogenic effect, a dose–response relationship has therefore been set depending on animal data with JECFA proposing lower limit on the benchmark dose for a 10% response ($BMDL_{10}$) for acrylamide to be 0.31 mg/kg body weight per day in case of induction of mammary tumors in rats and 0.18 mg/kg body weight per day for Harderian gland tumors in mice (JECFA, 2011). It therefore follows that the risk evaluation should be done by using the MOE approach which means that the ratio between the BMDL and the estimated dietary exposure is obtained.

The tendency to consume out of home foods and in-between meals such as potato crisps vary with regions and for more than a decade it has been on the increase taking important position as far as dietary nutrition is concerned (Guthrie et al., 2002). Although there is an increasing trend towards consumption of crisps in Kenya (Abong', et al., 2010), it is not clear as to what extent the consumers are exposed to acrylamide. Since acrylamide has been proven to be genotoxic and carcinogenic, possible risk may not to be wished off. The purpose of this study was to assess the level of exposure to acrylamide due to consumption of potato crisps in Nairobi, Kenya and propose mitigation measures necessary for its reduction.

4.1 Assessment of exposure to acrylamide and risk characterization

4.1.1 Materials and Methods

4.1.1.1Potato crisps consumption data

Due to limited information on potato crisps consumption in Kenya, consumption data were collected from retail outlets in Nairobi, Kenya, based on potato crisps consumers. Nairobi is the capital city of Kenya and it was purposively selected due to the large number of supermarkets and has the most crisps processing companies and hence a large population of crisps consumers. This study was carried out between February and April 2013 and it involved a cross-sectional survey applying quantitative data collection methods. Sampling was carried out according to Abong et al. (2010). A total of 315 consumers were available for interview from randomly selected retail outlets. Data was collected using a structured seven day recall questionnaire which had previously been pre-tested with 10 consumers (Appendix1). Data was collected on gender of consumer, frequency of consumption level, the preferred brand, estimated weight and height, and flavor, and trends in consumption. The daily crisps consumption was calculated by dividing the weekly intake (kg/person) by a factor of 7, and by the estimated body weight of an individual or an average weight of 60 kg where the weight was not available (JECFA, 2011).

4.1.1.2 Determination of acrylamide contamination of potato crisps

Sampling of potato crisps for analysis

Potato crisps for acrylamide contamination evaluation were sampled between February and March, 2013 from different retail outlets and street processors in Nairobi, Kenya. Forty three brands (35 were only salted while 8 were flavoured), which were sold in different retail outlets were purchased in duplicates of 100-200 g each, while fifteen unbranded samples were bought from five different street processors. Utmost care was taken to ensure that no similar brands were purchased from the same retail outlet.

Determination of acrylamide in crisps

Acrylamide contamination of potato crisps was determined following “Detection and Quantification of Acrylamide in Foods” method as described by the United States Food and Drug Administration (2002) using Gas chromatography (Shimadzu GC 14B, Japan) and Flame Ionization Detector (Shimadzu G-14B, Japan). Crisps samples were ground and 1g was combined with 10 ml of 0.1% formic acid solution and thoroughly mixed on a test tube shaker (KS250, Japan) for 5 minutes at 300 rpm. The samples were then centrifuged at 3000 rpm for 10 min to ensure easier removal of the oily top layer and solubility of the acrylamide. The supernatant was extracted then filtered using a filter paper (Wattman Paper No. 47) before being passed through a 0.45μm nylon syringe filter to remove any suspended particles and then stored in a refrigerator before cleanup and analysis stage.

Cleanup involved passing the filtered sample through a solid phase extractor tube (Carboprep tm 200 SPE tube, 6ml, 500mg) which was first activated by passing 2 ml of acetone solvent and then 2 ml of 0.1% formic acid. Water (1 ml) was first passed through the tube before the filtered sample solution was passed through the tube and allowed to flow under gravity. The SPE tube was then dried in a vacuum for one minute after which 2 ml of analytical grade acetone flowing through gravity was passed for elution. The elute was stored immediately in the refrigerator ready for the GC-FID analysis. The column used for chromatographic separation was Fused Silica Capillary (Supelcowax TM-10 Dimensions: 30 m X 0.53 mm, I.D. 0.5um film). The injection temperature was maintained at 260°C while nitrogen, the carrier gas, was supplied at

100 bars pressure. The linear velocity of the carrier gas was maintained at 62cm/sec at 100 °C, while the oven temperature was set at 100 °C then held for 0.5 min before it was allowed to increase at a rate of 15 °C/min to attain a final temperature of 200 °C . A calibration curve was built in the range of 50–1000 µg/kg from acrylamide standard (BDH Chemicals Analar), and measured values expressed as µg/kg for potato crisps. The analysis was carried out in the Department of Food Science and Technology, Jomo Kenyatta University of Agriculture and Technology. The limit of detection for the GC-FID was found to be 4.5ppm while the Limit of quantification was 45ppm.

4.1.1.3 Assessment of acrylamide exposure using probabilistic approach

Using @Risk TopRank 6 risk analysis software for excel (Palisade, UK), data for contamination and consumption were fitted to obtain the best distributions. All the formulae for distributions and outputs are indicated in Table 4.1. Independent consumption data were combined with acrylamide contamination results and exposure calculated. The average exposure and 95 percentile (P95) were obtained and compared with $BMDL_{10}$ to obtain MOE for risk characterization. To cater for variability and uncertainty, Monte Carlo simulation of exposure was performed using @Risk risk analysis software which combined randomly the consumption and contamination distributions. One million iterations were performed to describe the variability.

Table 4.1: Formulae used in quantitative risk assessment model for acrylamide in potato crisps

	A	B	C
	Unit	**Distribution**	**Formula**
1	Acrylamide , non-branded crisps (µg/kg)	Input (Contamination)	RiskExtvalueMin (8539.94, 1247.34)
2	Acrylamide , Overall Conatmination (µg/kg)	Input (Contamination)	RiskTriang (0.0, 16287)
3	Acrylamide , Branded crisps (µg/kg)	Input (Contamination)	RiskExpon (4261.5, 48.98)
4	Consumption (kg/kg bw/day)	Input (Extent)	RiskPearson5 (1.899, 0.0)
5	Acrylamide intake (µg/kg bw/day), non-branded crisps	Output	RiskOutput () + B4*B1
6	Acrylamide intake (µg/kg bw/day), overall	Output	RiskOutput () + B4*B2
7	Acrylamide intake (µg/kg bw/day), branded crisps	Output	RiskOutput () + B4*B3
8	Margin of Exposure (MOE)	Output	BMDL10/Intake

4.2 Results and discussion

4.2.1 Potato crisps Consumption Survey

Most of the people who consume potato crisps in Nairobi were mainly female as indicated in the figure two below as opposed to men. This is because women prefer sweet foods as compared to men who prefer foods that are filling.

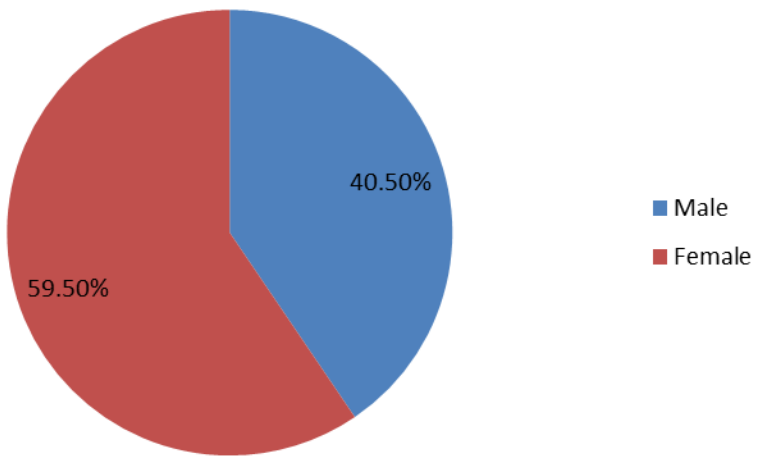

Figure 2: Gender of respondents

The educational levels of the respondents varied with 37.9% being in tertiary institutions, 37.8% from graduates of secondary ordinary level while 18.9 % had secondary advanced level of education. Figure three below indicates the education levels of the respondents interviewed who purchase and consume potato crisps in Nairobi.

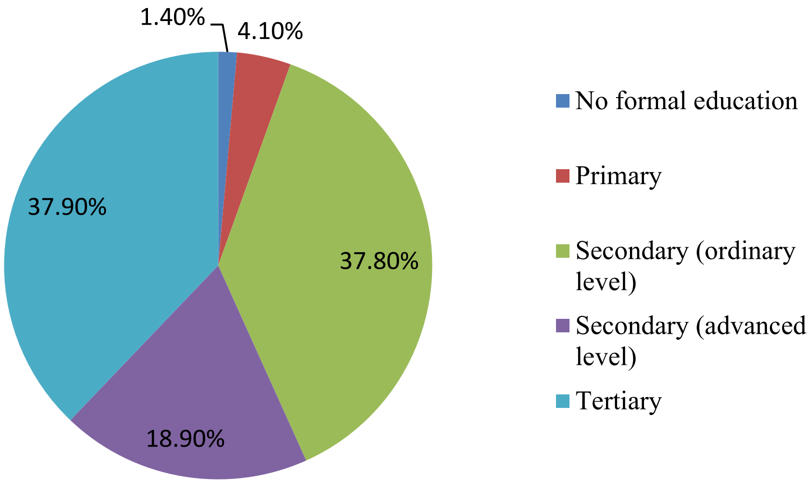

Figure 3: Education levels of respondents

About 51.9 % of those interviewed were students while 32.9% were employed and received a monthly salary. Only 5.1 % were not employed while those who were self-employed and casual laborers had a 2.5% representation. Nairobi city has many colleges and schools and this explains why most respondents (crisps consumers) were students. Crisps can be consumed as a snack of choice either in between meals or some people do consume it as a meal on its own. Figure 4 indicates graphical representation of occupation of the crisp consumers in Nairobi.

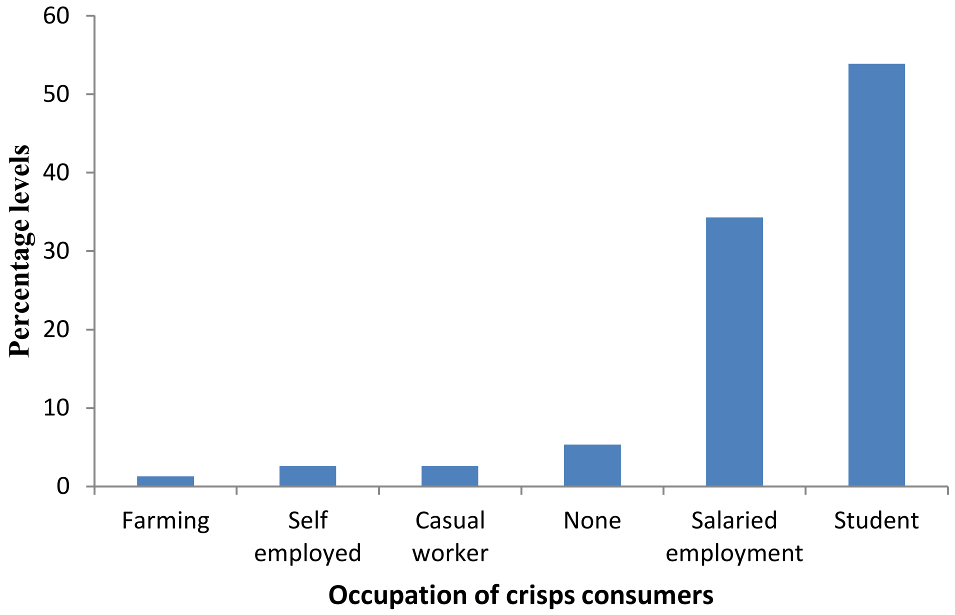

Figure 4: Occupation of the respondents crisps consumers

The age of the respondents ranged from 5 years to 51 years with majority being in the age bracket of 20-24 years who had a percentage of 38%, followed by 25-29 years with 22.7%. 89% of those interviewed were between the ages of 17 to 35 years. This is an indication that the highest consumers of the potato crisps are young people and thus in agreement with the findings of Abong *and others* (2010) who observed that most crisps consumers are young people. This may result in a higher intake of acrylamide among the younger generation and hence a potential risk of developing cancer related disorders. Figure five indicates the consumption pattern of crisps based on age by the consumers in Nairobi.

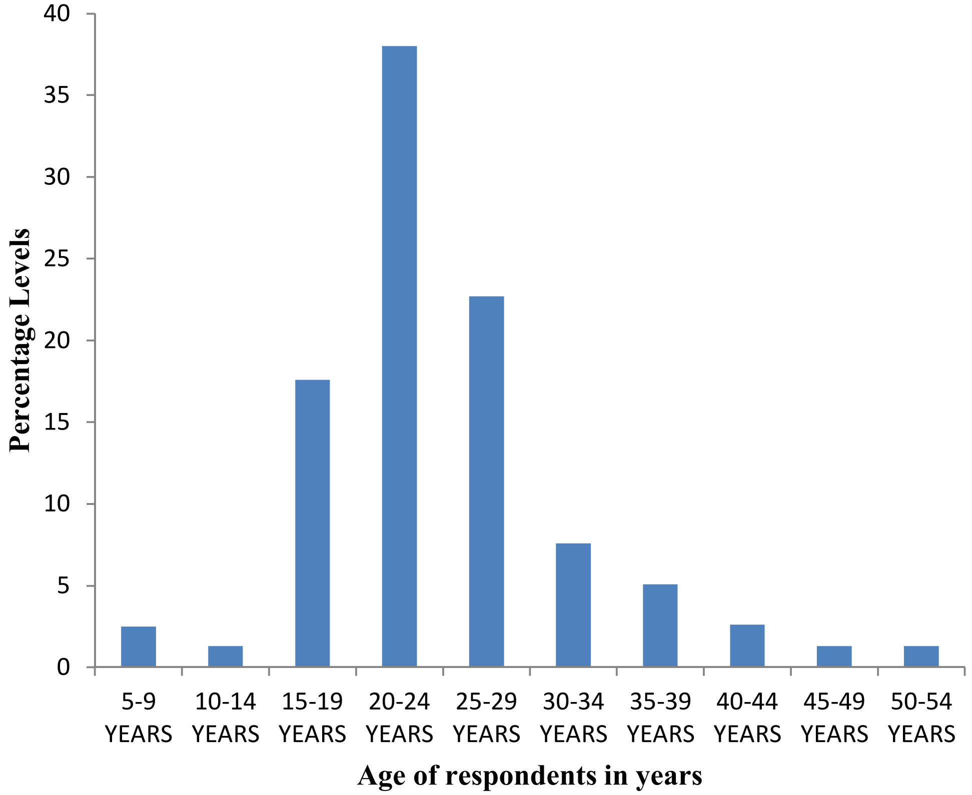

Figure 5: Age of respondents of crisps consumers in Nairobi

The most purchased unit package was 100g (40.5%); followed by 50g 25.2% and then 30g (12.7%). 200g were purchased by 11. 4%. The least purchased package unit was 70g (3.8%).

Approximately 92.1% of the respondents interviewed purchased their potato crisps from the supermarket with only 7.9% purchasing the crisps from the kiosks. The reasons given for preference of place of purchase included high quality (72%), proximity (12%), affordability (9.3%) and need for more quantity (6.7%).

Figure 6 indicates the frequency of consumption of potato crisps expressed as percentages.

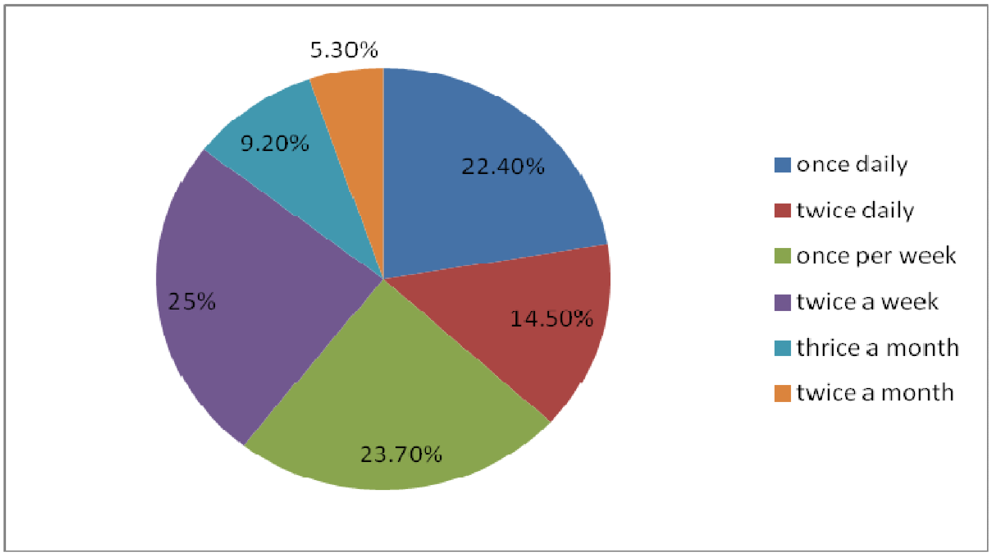

Figure 6: Frequency of potato crisps consumption by respondents.

The reasons given for the choice of flavours consumed included: irresistibility, taste, and delicious nature, quality of the product and the crunchy nature of the potato crisps. The brands that were preferred by the respondents were Amigos (27.8%), Tropical Heat (21.5%), Deepys (8.9%), Chigs (2.5%), Eskay (1.3%), Urban Bites (1.3%), Hoops (1.3%), Yankee Doodles (1.3%), Wow (1.3%), Krackles (1.3%), Kripsii (8.9%), Happys Golden (1.3%), Pringles (1.3%) and Potato crisps (6.3%).

Figure 7 shows preference in consumption based on flavour of the potato crisps.

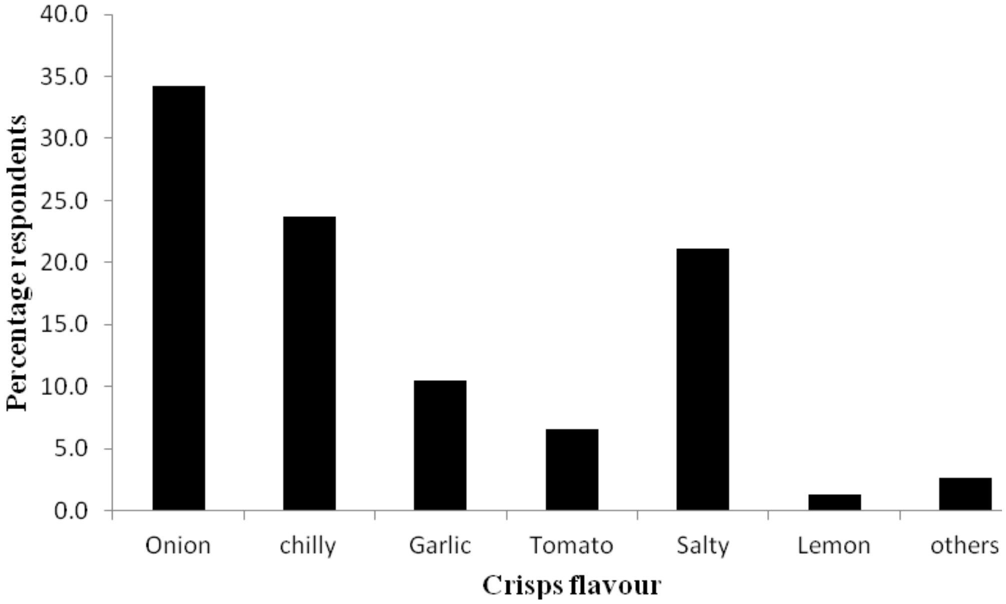

Figure 7: Percentage consumption based on the flavour of potato crisps

About 81% of the respondents interviewed had no complains about the crisps they consume while those who had complains majorly stated the following reasons: the quantity packaged being quite less compared to the price charged; some crisps in the packages being broken down into tiny pieces; high fat and salt content. Despite these complaints, 59.5% noted that their consumption rate was increasing due to the sweet taste, increased income and the sustainability of the potato crisps. Some respondents (27.9%) indicated that their consumption was decreasing due to increased cost of living, high fat and salt content due to related health complications and the high pricing of the potato crisps.

4.2.2 Acrylamide contamination, consumption and exposure

Table 4.2 shows summary results of distribution fit for acrylamide contamination and consumption as well as simulations for acrylamide intake in potato crisps, both consumption and contamination being defined with continuous distributions. Potato crisps contamination with acrylamide was defined with an extreme value minimum distribution with a mean of 7819.96µg/kg for non-branded crisps from street processors, while an exponential distribution characterized contamination of branded crisps mainly from supermarkets with a mean of 4212.53µg/kg. Overall contamination was defined with a triangular distribution with mean of 5429 µg/kg. On the other hand, consumption data followed pearson5 distribution with mean value 3.0 x 10^4 kg/kg bw/day. Significantly higher contamination values were found in unbranded crisps samples compared to branded samples from retail outlets.

Table 4.2: Results of Distribution fitting and simulations of acrylamide intake in potato crisps

Unit	Output (Mean)	Min-Max
Acrylamide , non-branded crisps (µg/kg)	7819.96	-8814.95-11834.47
Acrylamide , Overall contamination (µg/kg)	5429.00	0.001-16273.16
Acrylamide , Branded crisps (µg/kg)	4212.53	-48.98-72667.54
Consumption (kg/kg bw/day)	0.00	0.00-0.3482
Acrylamide intake (µg/kg bw/day), non-branded crisps	2.25	-8.22-2059.49
Acrylamide intake (µg/kg bw/day), overall consumption	1.57	0.00-1744.13
Acrylamide intake (µg/kg bw/day), branded crisps	1.22	-0.67-4198.21

The levels of acrylamide contamination in the current study seem to be quite higher compared to the values reported by other researchers in Italy (27-1400 µg/kg) by Tateo et al. (2010), Sweden (30-2300 µg/kg) by Tareke et al. (2002), Iran (244-1688 µg/kg) by Boroushaki et al. (2010), France (954 µg/kg) by Sirot et al. (2012), and Chile (40-1770 µg/kg) by Pedreschi at al.

(2010).Variation of acrylamide in potato crisps is influenced by a number of factors ranging from agronomic practices, storage conditions to processing parameters (De Meulenaer et al., 2008). Potato variety plays a major role in determining the levels of reducing sugars and have been shown to have direct relationship with acrylamide (Williams, 2005; Amrein et al., 2007; Abong', 2009; Claeys et al., 2010). Post-harvest handling and storage conditions have profound effect on the levels of reducing sugars. Storage at temperatures below 8^{o}C generally leads to accumulation of reducing sugars which requires tubers to be reconditioned before frying to achieve low acrylamide contamination and desired color. This is, however, cultivar dependent (Abong', 2009).

Pre-frying activities such as soaking in water, blanching and coating have been shown to reduce acrylamide in crisps due to leeching and reduce maillard reaction necessary for acrylamide formation (Matthaus et al., 2004; Zhiqiang and Scanlon, 2007; Pedreschi et al., 2010a). On the other hand, frying at elevated temperature for long duration are major facilitators to acrylamide contamination (Kita et al., 2004; Cummins et al., 2008). The size of the product may also influence contamination level, tends to be higher in thin slices or sticks (Biedermann et al., 2010). Acrylamide contamination seems generally high in Kenyan potato crisps, a scenario that may be attributed to many factors. The use of any available potato tuber by processors especially from the streets (Abong' et al., 2010) and use of immature tubers (Haase et al., 2004) may be significant contributor. Uncontrolled frying temperature which is a common scenario in Kenya is no doubt another contributor to higher acrylamide contamination. Branded crisps sold in the retail outlets come from small-medium to large enterprises some of which have raw material and processing control while majority lack the control measures.

All consumers in the current study were exposed to acrylamide, the level increasing with consumption. The mean acrylamide exposure for overall crisps contamination was found to be 1.57µg/kg bw/day, while the 95th (P95) percentile was 5.1µg/kg bw/day (Table 4.3).

Table 4.3: Estimated Margins of Exposure (MOE) for the mean and 95th percentile of dietary exposure to acrylamide of crisps consumers in Nairobi, Kenya.

	Overall crisps		**Branded crisps**		**Non-branded crisps**	
	Mean	**P95**	**Mean**	**P95**	**Mean**	**P95**
Dietary exposure (µg/kg bw/day)	1.57	5.10	1.22	4.24	2.26	6.54
MOE ($BMDL_{10}$=180 µg/kg bw/day)[1]	115	35	148	42	80	28
MOE ($BMDL_{10}$=310 µg/kg bw/day)[1]	197	61	254	73	137	47

[1] $BMDL_{10}$ defined for carcinogenic and toxigenic effects (JECFA, 2011).

The acrylamide intake was higher in non-branded crisps with a mean value of 2.26 µg/kg bw/day and 95th percentile of 6.54 µg/kg bw/day. The intake is dependent on contamination and consumption levels (Arribas-Lorenzo and Morales, 2009). High contamination of potato crisps could be the major contributor to the current high exposure to acrylamide by crisps consumers in Nairobi, Kenya. Together with roasted coffee, biscuits and French fries, potato crisps have been identified at the international level to be a major contributor to acrylamide exposure (EFSA, 2011; JECFA, 2011; Katz, et al., 2012; Sirot et al., 2012). The mean exposure in the current study was quite high when compared to 0.40 µg/kg and 0.43 µg/kg respectively reported by Mestdagh et al. (2007) and Sirot et al. (2012) which considered total diet studies in Belgian and French populations, respectively. The current exposure levels are, however, quite high when compared to other research findings when only potato crisps is taken into account (Table 4.4).

Table 4.4: Acrylamide intake (µg/kg bw/day) from total diet studies and potato crisps reported in literature.

Food	Reported mean	Difference	Country	Author
Total diet	0.430	1.140	France	Sirot et al., 2012
Potato crisps	0.010	1.560		
Total diet	0.350	1.220	Belgium	Claeys et al., 2010
Potato crisps	0.100	1.470		
Total diet	0.400		Belgium	Mestdagh et al., 2007
Potato crisps	0.068	1.502		
Total diet	0.400	1.170	Netherlands	Bongers et al., 2012
Potato crisps	0.047	1.523		
Potato crisps	0.042	1.528	Spain	Arribas-Lorenzo and Morales, 2009
Total diet	0.400	1.170	USA	FDA, 2006
Potato crisps	0.045	1.525		
Total diet	0.441	1.129	USA	Katz et al., 2012
Potato crisps	0.080	1.490		

4.2.3 Risk characterization

As unavoidable food contaminant that is also carcinogenic and genotoxic, a Margin of Exposure (MOE) has been proposed and adopted as a useful concept giving indication of possible extent of a given risk (EFSA, 2005; JECFA, 2005). This is because MOE compares the lower limit on the benchmark dose for a 10% response ($BMDL_{10}$) and the exposure. It therefore follows that the higher it is, the lower the risk of a given contaminant and vice versa. The calculated MOE for the mean were respectively 115 and 197 for both $BMDL_{10}$ of 0.18 and 0.31 mg/kg bw/day while for 95th percentile were respectively 35 and 61. The MOE for the 95th percentile for non-branded potato crisps was extremely low (28). Since JECFA (2011) considers MOEs of 45-310 to be of health concern, the current values of the mean and at the 95th percentile would equally raise serious safety concern for crisps in Kenya. On the same note, if the conclusions of EFSA/WHO

(2005) which places any MOE lower than 10,000 to be of public health concern, then the level of exposure in this study raises in general great concern that require urgent redress. More efforts are therefore required to reduce acrylamide as a process contaminant in Kenyan potato crisps.

4.3 Mitigation strategies

Being a probable carcinogen and neurotoxic, it is generally agreed that efforts geared towards development of for acrylamide reduction strategies ought to be promoted (JECFA, 2011). A concerted effort should run through the chain from farm to table in order to keep acrylamide exposure as low as reasonably achievable (ALARA) based upon current knowledge (FDE, 2011). Selection of cultivars with low reducing sugar levels and asparagine for crisp production will no doubt be a major step towards reduction of acrylamide contamination and subsequent exposure (Putz 2004). This is especially so because reducing sugars are the limiting factors in acrylamide formation (Becalski et al., 2004; Williams, 2005). Maturity and harvest date have been indicated to have a major influence on tuber sugar content (Haase and Weber, 2003). Immature or early harvested tubers result in high reducing sugar levels (Haase et al., 2004). Harvesting mature tubers will definitely contribute significantly to reduction of acrylamide. Government intervention through extension services and promotion of appropriate marketing structures is of necessity.

Storage of potato tubers at lower than 8°C over longer duration leads to increased reducing sugars, and hence high acrylamide contamination. Where such storage is done, reconditioning should be carried out first before processing while taking into account the cultivar in question.

Blanching and soaking of potato slices in solutes such as sodium chloride have as well been identified as important technological risk reduction as they leech out reducing sugars (Matthaus et al., 2004; Pedreschi et al., 2004; Cummins et al., 2008; Pedreschi et al., 2010a). Blanching should, however, be chosen depending on the cultivar and having consideration on impacts on texture, oil content, color and flavor the texture and flavour of the fried product (Zhiqiang and Scanlon, 2007), and increases the oil content (Pedreschi and Moyano, 2005). Addition of ingredients that inhibit acrylamide formation such as acetic acid (Mestdagh et al., 2008), protein hydrolysates, amino acids and multivalent ions can be promoted during processing to reduce acrylamide.

Apart from the structured processors the responsibility of street processors in the mitigation of acrylamide exposure through reduction of acrylamide in their prepared products is critical. Frying time and temperature have been shown to have significant influence on acrylamide ((Becalski et al., 2003; Stadler and Scholz, 2004; Cummins et al., 2008). For instance, decreasing frying temperature of crisps from 185 to 175^{o}C has been shown to reduce acrylamide contamination by 35% (Cummins et al., 2008). Low to moderate temperatures of frying and short time will reduce production of acrylamide (Kita et al. 2004). Awareness creation and proper control by ministry of public health through policy guidelines will be helpful in this respect. Adoption with time of concepts such as signal or indicative values or action limits such as those taken by European Commission may be adopted to help policy makers and encourage processors to take action. Consumers should also be guided on the optimum frying conditions necessary for low acrylamide production.

Nutritional education can also play significant role in reducing acrylamide exposure. Consumption of a nutritionally balanced meal composed of foods from many sources and rich in fruits and vegetables have been shown to significantly reduce acrylamide exposure to a large population of out of home eating consumers (Mestdagh et al., 2007; Maillot et al., 2010). The catering and processing units have then to become important partners in order to promote balanced diets as has been shown to in success stories in Europe (Lachat et al., 2005). Moderate consumption of potato crisps should be encouraged as a responsibility of the consumer (Slayne et al., 2005).

4.4 Conclusions and Recommendations

High contamination of potato crisps increases exposure to acrylamide by consumers in Nairobi, Kenya. The exposure is higher for consumers of street processed crisps which subsequently lead to lower Margin of Exposure indicating that such consumers may be at a potential risk. The current situation therefore requires urgent attention and concerted efforts to reduce the high exposure. The current study only focused on potato crisps consumers and did not consider acrylamide contribution from total diet and seasonal variation. It also did not include children who may be more exposed. Thus more study need to be done in these two areas.

CHAPTER FIVE

Effect of Variety, Frying Temperature and Slice Thickness on Colour and Acrylamide in Potato Crisps from Kenyan cultivars

Abstract

Acrylamide, a chemical found in several carbohydrates rich foods processed at high temperatures, has been classified as a potential human carcinogen; the need to minimize its presence in human diet cannot be overemphasized. This study was designed to determine how acrylamide formation is influenced by potato varieties, processing temperatures and slice thickness in potato crisp produced from established Kenyan varieties.

Four potato varieties (Kenya Mpya, Sheherekea, Tigoni and Dutch Robjin) were purposively selected due to their suitability for crisp production and were planted at Kari Tigoni. The potato tubers were sliced into three slice thicknesses (1-0mm, 1.5mm and 2.00mm) and subjected to three frying temperatures of 160°C, 170°C and 180°C). The dry matter of the tubers ranged from 21.5% in Kenya Mpya to 28.4% in Sherehekea. The fructose levels ranged from 0.068% in Tigoni to 0.241 % in Kenya Mpya; glucose levels ranged from 0.155% in Tigoni to 0.218% in Kenya Mpya; Sucrose levels from 0.606% in Sheherekea to 0.868% in Kenya Mpya. In the potato crisps Kenya Mpya variety fried at 160°C of thickness 2.0mm had the highest moisture content of 2.575%. Most of the crisps from the four varieties processed at different temperatures and thicknesses were light colored with lightness (L*) parameters greater than 50 and towards red as shown by positive values of redness parameter (a*) indicating that there was excess browning of the products during frying. Acrylamide levels significantly ($P \leq 0.05$) differed between the varieties and ranged from 3129 ppb in Dutch Robjin to 13480ppb in Kenya Mpya. There was a significance difference (P0.05) in acrylamide levels Pwith temperatures of 180°C resulting in higher acrylamide content compared to those of 160°C and 170°C. Similarly the slice thickness of 2.0mm had high values of acrylamide levels and the redness parameter (a*) too. There was a strong correlation between acrylamide formation and glucose (r=0.761) and fructose (r=0.44) formation.

Key words: Acrylamide, reducing sugar, slice thickness, frying temperature, variety

5.0 Introduction

Potatoes (*Solanum tuberosum)* are a cheap, readily available, high carbohydrate food perceived by consumers as an important component of a healthy, balanced diet. The potato represents one of the world's major staple food crops and is the most widely grown food crop after rice, wheat and maize globally (CIP, 2013). In Kenya, for example, potato is the second most valuable cash and food crop after the cereal grains (MoA, 2009). Potato tubers that are round in shape, lower reducing sugar contents and higher dry matter have been shown to be suitable for crisps processing for most processors because they easily make the required crisp sizes (Kulkarni and Govinden, 1994; Kabira and Lemaga, 2006; Torres & Parreño, 2009).

Acrylamide has been found to be formed in starchy foods heated at higher temperatures either through frying or baking. The maillard reaction between the amino acid asparagine and reducing sugars or reactive carbonyls at temperatures above 120°C has been suggested as possible pathway for its formation in these foods (Mottram et al., 2002; Tarek et al., 2002). The sugars and asparagine levels vary with variety and hence the influence on acrylamide.

Factors that influence acrylamide formation include processing temperature, time, content and species of reducing sugars and amino acids, pH, moisture content and frying oils, indicating that acrylamide in foods can be decreased by changing processing technology (Ciesarova et al., 2006).

According to Medeiros Vinci et al. (2012) there is little correlation between asparagine content alone and acrylamide formation. De Wilde et al. (2006) reported that acrylamide levels in fried potatoes derived from 16 different varieties correlated to reducing sugar content of the potatoes ($R^2 = 0.82$, $n = 96$). In addition, reducing sugar contents below 1 g kg^{-1} fresh weight has been taken as indicative of a suitable processing quality (Biedermann-Brem et al., 2003). Asparagine concentrations are relatively in excess compared to reducing sugar contents. Thus, reducing sugars represent the limiting factor in acrylamide formation and therefore will largely determine acrylamide formation in potato products (Amrein et al., 2003; Becalski et al., 2004; De Wilde et al., 2005; Medeiros Vinci et al., 2010).

The acrylamide content in chips showed a high correlation with glucose content in the tubers (r^2=0.884, n=20). A similar correlation was observed for fructose (r^2=0.884, n=20).

Since acrylamide is formed towards the end of frying, the temperature during the second half of the process is more important than that regulated by the thermostat. Taubert and others (2004) found that acrylamide levels increase with greater temperature and cooking time in potatoes with low surface-to-volume ratios (SVR), whereas for potatoes with high surface-to-volume ratios acrylamide levels peak and then decline with further heating. Variation may be a function of the availability of substrate; in low SVR potatoes, there is a larger, steady supply of sugars and asparagine, but in high SVR potatoes, the supply is more quickly depleted.

Investigations by several researchers have established existence of a relationship between the acrylamide content and surface colour, as evaluated by the standard CIE L*a*b* parameters or by computer vision. A good correlation between the acrylamide content of fried potatoes and their colour has been shown, though it was observed that this correlation was less close for large surface-to-volume material, such as potato crisps, in comparison with small surface-to-volume material, such as French fries (Pedreschi et al., 2005; Pedreschi et al., 2006; Pedreschi et al., 2007). This study was thus designed to determine the effect of processing temperature, slice thickness and potato variety on the colour and acrylamide content.

5.1 Materials and Methodology

5.1.1 Potato for processing

Four potato varieties (Dutch Robjin, Sherehekea, Kenya Mpya and Tigoni) known to be suitable for processing potato crisps (Abong' et al., 2010) were grown under standard cultural conditions at the National Potato Research Centre (KARI), Tigoni. Harvesting of potatoes was done at maturity followed by curing in dark store for two weeks. Processing was carried out and acrylamide levels, moisture content and color determined immediately after processing. Before processing tubers from each variety were analyzed for reducing sugars and dry matter content.

5.1.2 Crisps processing

About ten potato tubers from each variety were randomly selected from a net bag and peeled using a knife and sliced into 3 sizes of 1, 1.5 and 2 mm thickness. The slices were washed to remove surface starches then dried with a clean cloth towel, and duplicate 200 g samples fried in sunflower oil maintained at fixed temperatures of 160, 170, 180 ^{0}C till ready. The crisps were then removed from oil, drained for 30s and turned into a clean tray, cooled and packaged into labelled plastic bags and sealed before analysis.

5.1.3 Experimental design

Samples were prepared in accordance with a full factorial experimental design. The design parameters were: potato variety (4 levels: Dutch Robjin, Sherehekea, Kenya Mpya and Tigoni), frying temperature (3 levels: 160, 170 and 180 °C), and slice thickness (3 levels: 1.0mm, 1.5mm and 2.0mm). Statistical analysis was carried out using SAS version 9.1.3 to determine both inferential and descriptive statistical tests including Analysis of Variance and least significant difference test (LSD) for the variables. Pearson correlation analysis and multiple regression analysis were also performed to determine relationships between acrylamide, colour parameters, moisture and reducing sugars. Where the differences of $p \leq 0.05$ existed, the samples were considered to be significant.

5.1.4. Materials and standard preparation

Acrylamide (2-propene amide) [CAS No. 79-06-1] (>99.5%) was obtained from Kobian house (Mombasa road, Nairobi, Kenya). Individual stock of acrylamide solutions were prepared by dissolving 100 mg of compound in 100 ml of acetone and stored in a glass-stoppered bottle at 4 °C. Standard working solutions at various concentrations 800ppm, 400ppm, 200ppm, 100ppm and 50ppm were daily prepared by appropriate acetone dilution of stock solution aliquots. Analytically grade acetone (2-propanone) of molecular formula $(CH_3)_2CO$ [CAS No.67-64-1] used in GC-FID and Acetonitrile of molecular formula C_2H_3N [CAS No.75-05-8] for HPLC were obtained from Kobian House Nairobi.

5.2 Analytical methods

5.2.1 Determination of dry matter content of raw potato tubers

Four tubers were randomly selected from each of the four varieties. The tubers were then cut into small slices (1-2 mm) and mixed thoroughly. Dry matter contents were determined by drying triplicate 5g at 105^{o}C for 4 hours in a hot air oven (DSO-500D, Israel).

5.2.2 Extraction and determination of reducing sugars contents of the raw potato tubers

Reducing sugars in raw tubers were determined by HPLC through modification of the method described by Abong' *et al.,* (2011). Approximately 10 g of homogenized potato slices were weighed into a 250 ml conical flask and 50 ml of 96 % alcohol were then added and mixed well. The mixture was then refluxed at 100°C for 1 hour, stirring occasionally. The resultant slurry was then filtered and the filtrate collected. The conical flask was then rinsed 3 times with 5 ml of 80 % alcohol. The filtrate was then transferred into 150 ml pear-shaped flask and the solvent evaporated to dryness at 60°C using a rotary vacuum evaporator (RE200, England, United Kingdom). Approximately 10 ml of distilled water was added to the dried sample. Afterwards, the dissolved sample was placed in duplicates of 2 ml into a test tube and 2 ml of diethyl ether added. The mixture was thoroughly shaken and allowed to stand before removing the ether layer. This was repeated 3 times. Excess ether was then flashed off using a vacuum (Heraeus, RVT 360, Germany). Equal amounts of acetonitrile was then added to the samples before being stored at 5°C ready for determination of sugars using HPLC. The samples were micro-filtered to remove any debris before injecting 20 µl into a HPLC, SCL-10A (Shimadzu, Tokyo, Japan) fitted with a Refractive Index Detector, RID-6A (Shimadzu, Tokyo, Japan). Chromatographic conditions which included a mobile phase of acetonitrile: water (80:20) pumped through a

and Flame ionizable detector (G-14B,Shimadzu, Japan) with a chromatopac (C-R8A, Shimadzu, Japan) and the aspirator (GP-5-254-SI).

Acrylamide extraction

The samples were crushed using a pestle in a mortar and approximately 1g was weighed before addition of 10 ml of 0.1% formic acid solution. The mixture was then mixed on a test tube shaker (KS250, Japan) for 5 minutes at 300 rpm followed by centrifugation (H 20000,Tokyo, Japan) at 3000 rpm for 10 min to ensure easier removal of the oily top layer and ensure further solubility of the acrylamide compound specs of centrifuge. The supernatant was extracted then filtered using a filter paper (Wattman paper No. 47) before being passed through a 0.45µm nylon syringe filter to remove any suspended particles and then stored in a refrigerator awaiting the clean up and analysis stages.

Clean up stage

Clean up involved passing the filtered sample through a solid phase extractor tube (Carboprep tm 200 SPE tube, 6ml, 500mg) which was first activated by passing 2 ml of acetone solvent and then 2 ml of 0.1% formic acid. The filtered sample solution was then passed through the tube and allowed to flow under gravity. Water (1 ml) was then fast passed through the tube. The SPE tube was then vacuum dried for one minute after which 2 ml of analytical grade acetone flowing through gravity was passed for elution. The elute was stored immediately in the refrigerator ready for the GC-FID analysis. 1 microlitre of the elute was later injected into the column and the chromatogram obtained and later interpreted.

Gas chromatography conditions

The column used was a supelcowax capillary column since it gave good results during determination of the suitable condition for analysis using this column. The injection temperature was maintained at 260°C while nitrogen, the carrier gas, was supplied at 100 bars pressure. The linear velocity of the carrier gas was maintained at 62cm/sec at 100 °C, while the oven temperature was set at 100 °C then held for 0.5 min before it was allowed to increase at a rate of 15 °C/min to attain a final temperature of 200 °C . The retention time of the acrylamide was found to be between 8.4 and 8.7 minutes. The limit of detection for the GC-FID was found to be 4.5ppm while the Limit of quantification was 45ppm.

5.3 Sensory evaluation of the processed potato crisps

Coded samples were presented to 10 panellists and who scored for colour, texture, flavour, oiliness and overall acceptability on a 7-point hedonic scale ranging from 1 (dislike very much) to 7 (like very much) according to Larmond (1977).

5.4 Data Control and Analysis

5.4.1 Data quality control

To ensure quality data was achieved: all analysis was done in triplicates or duplicates, equipment and instruments were calibrated daily before use to limit erroneous results. The standards used for acrylamide and reducing sugars determination were prepared under specified conditions. In addition, where time and temperature was critical in the analysis, these parameters were closely monitored to limit deviations. Data entry was done promptly, cleaning, and repetition where necessary to minimize errors and eventually obtain data of high quality.

5.4.2 Data analysis

Both descriptive and inferential statistical tests including analysis of variance (ANOVA), Duncan multiple range and least significant difference test (LSD) for the variables were carried out using the Statistical Analysis System (SAS) version 9.1.3. Pearson correlation analysis and multiple regression analysis were also performed to determine relationships between acrylamide and moisture and between acrylamide and colour parameters. Where the differences of $P \leq 0.05$ existed, the samples were considered to be significant.

5.5 Results and Discussion

5.5.1 Results

Figure 4 shows the percentage dry matter contents of the four Kenyan potato varieties used in this study.

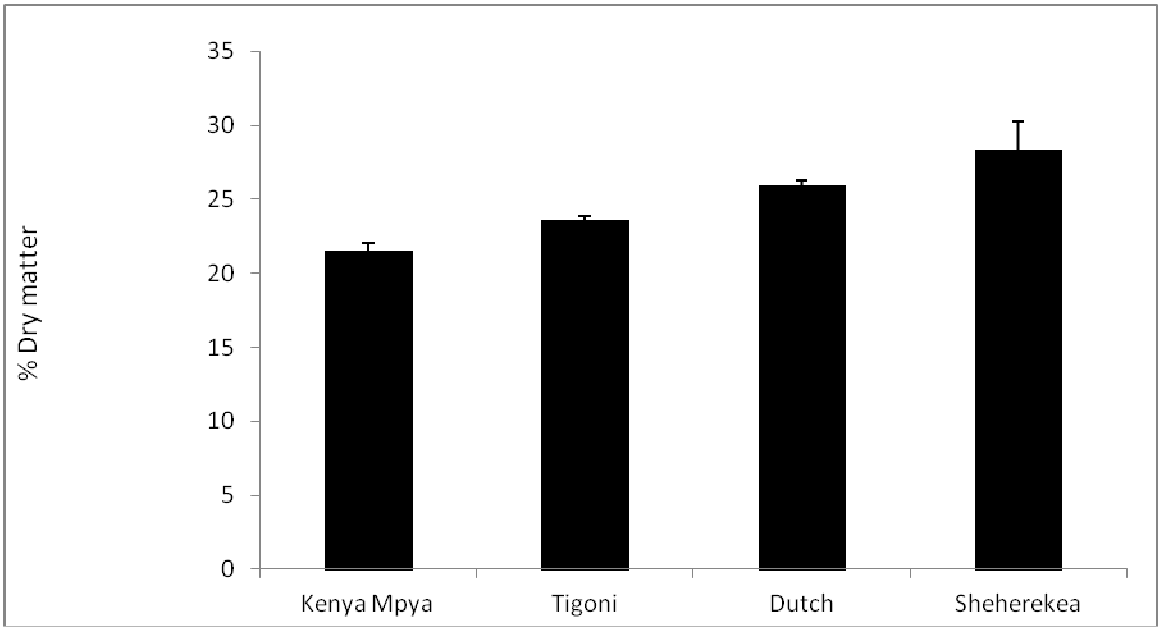

Figure 8: Dry matter content of four Kenyan potato varieties. The bars represent the standard error bars.

The tubers used for processing crisps had dry matter levels ranging from 21.5% (Kenya Mpya) to 28.4 % (Sheherekea) and there was a significant ($P \leq 0.05$) difference between the varieties.

There was a significant difference ($P \leq 0.05$) between the varieties Sheherekea, Tigoni and Kenya Mpya varieties. No significant difference ($P > 0.05$) existed between Sheherekea and Dutch Robjin and similarly between Dutch Robjin and Tigoni varieties. In addition no significance difference ($p > 0.05$) was observed in dry matter content between the varieties Tigoni and Kenya Mpya.

Table 5.1 indicates the levels sugars on dry weight basis expressed as g/100g from the four potato varieties.

Table 5.1: Sugar levels of the four potato varieties expressed in g/100g on dry weight basis

Variety	**Fructose**	**Glucose**	**Sucrose**
Kenya Mpya	0.241±0.001a	0.218±0a	0.868±0.001a
Dutch Robjin	0.147±0.001b	0.185±0a	0.679±0.002ab
Sherehekea	0.137±0.003c	0.155±0a	0.606±0.002ab
Tigoni	0.068±0.001d	0.161±0.003b	0.622±0.022b

Values are means of three determinations ± standard deviation.

Values with the same letters in the same column are not significantly different at 5% level of significance.

Kenya Mpya had the highest levels of sucrose, fructose and glucose while Tigoni had the lowest level of fructose and Sheherekea lowest levels of both sucrose and glucose. Fructose levels significantly differed significantly across all the varieties ($P<0.05$) with Kenya Mpya recording high levels of fructose of 0.241% compared to Tigoni which had 0.068%. The glucose levels were higher in Kenya Mpya variety (0.218%) and low in Sheherekea (0.155%). There was no significance difference ($P>0.05$) in glucose levels between Kenya Mpya, Dutch Robjin and Sheherekea but the three varieties differed significantly ($P\leq0.05$) with Tigoni in glucose levels. The levels of Sucrose ranged from 0.606% in Sheherekea to 0.868% in Kenya Mpya. No significant difference ($P> 0.05$) existed between Sheherekea, Kenya Mpya and Dutch Robjin but significant difference ($P\leq 0.05$) existed between Kenya Mpya and Tigoni in sucrose levels.

The moisture content of the processed potato crisps as affected by frying temp and slice thickness is given in Table 5.2.

Table 5.2: Moisture content of processed potato crisps from four Kenyan potato varieties

Variety	Frying temperature	Slice thickness	% Moisture content	Variety	Frying temperature	Slice thickness	% Moisture content
Dutch Robjin	160°C	1.0mm	0.431±0.09bcd	Sheherekea	160°C	1.0mm	1.501±0.05lm
		1.5mm	0.445±0.05c			1.5mm	1.305±0.04k
		2.0mm	1.348±0.14jklm			2.0mm	1.331±0.12jkl
	170°C	1.0mm	1.501±0.03lm		170°C	1.0mm	1.162±0.16hijk
		1.5mm	0.433±0.09bcd			1.5mm	1.099±0.08hij
		2.0mm	1.531±0.10lmn			2.0mm	1.03±0.09hi
	180°C	1.0mm	0.892±0.07fgh		180°C	1.0mm	1.192±0.18ijk
		1.5mm	0.987±0.07ghi			1.5mm	0.986±0.16ghi
		2.0mm	0.866±0.11fgh			2.0mm	1.071±0.16ghi
Kenya Mpya	160°C	1.0mm	1.856±0.06op	Tigoni	160°C	1.0mm	0.595±0.24bcde
		1.5mm	1.872±0.07opq			1.5mm	0.572±0.18cdef
		2.0mm	2.575±0.67pqr			2.0mm	0.952±0.20fghi
	170°C	1.0mm	1.289±0.13jkl		170°C	1.0mm	0.82±0.33cdefgh
		1.5mm	1.433±0.16klm			1.5mm	0.898±0.28efghi
		2.0mm	1.514±0.08lm			2.0mm	0.878±0.20efghi
	180°C	1.0mm	1.112±0.13hij		180°C	1.0mm	0.382±0.00b
		1.5mm	1.187±0.08ij			1.5mm	0.243±0.18a
		2.0mm	1.096±0.08hij			2.0mm	0.465±0.16bcde

Values are means of three determinations ± standard deviation.

Values with the same letters in the same column are not significantly different at 5% level of significance.

The moisture content ranged from 0.243% in crisps processed from the Variety Tigoni fried at 180°C and slice thickness 1.5mm to 2.575% from Kenya Mpya variety fried at 160°C and slice thickness 2mm. Highest moisture content was reported in potato crisps processed from Kenya Mpya variety and the lowest levels in those processed from Tigoni.

The moisture content of the processed crisps showed a significant difference ($P \leq 0.05$) between varieties with Kenya Mpya recording the highest moisture content and Tigoni the lowest moisture content. The higher the temperature of frying the lower the moisture content as potato crisps processed at temperatures of 180°C attained the lowest moisture content compared to those that were fried at 170°C and 160°C. There was a significance difference ($P \leq 0.05$) in moisture content between crisps processed at 180°C and those processed at 170°C and 160°C, though no significance difference ($P > 0.05$) existed between those processed at 160°C and 170°C.

Similarly the greater the slice thickness the higher the moisture content as the slice thickness of 2.0mm had the highest moisture content and significantly differed ($P \leq 0.05$) with those from the slice thicknesses of 1.0mm and 1.5mm. The moisture content of the potato crisps with slice thicknesses of 1.0mm and 1.5mm showed no significance difference at 95% confidence interval.

The color parameters of crisps processed from the four cultivars are given in Tables 5.3

Table 5.3: Color of crisps processed from the four potato varieties.

Cultivar	Frying temperature	Slice Thickness	L*	a*	b*
Dutch Robjin	160°C	1.0mm	49.7±3.5bcdef	1±0.9ab	23.6±0.6ef
		1.5mm	51.7±4.3cdefg	4±4.1abcdefgh	26.7±1.1ghi
		2.0mm	51.3±3.7cdef	8.4±3.4efghijk	29.1±3.4ghijklm
	170°C	1. 0mm	56.2±3.8efghi	3.1±1.9abcde	23.5±2defg
		1.5mm	51.4±3.1cdef	7.6±2.4efghi	28.±0.4ij
		2.0mm	53.5±5.1cdefgh	10±1.5hijk	31±1.5jklm
	180°C	1.0mm	53.3±5.5cdefgh	1.1±1ab	28.4±3.2ghijkl
		1.5mm	58.5±1.8ghi	1.7±0.3b	34.5±0.5o
		2.0mm	63.7±5.7hijklmn	1.8±1.2abc	34.3±1.4mnop
Kenya Mpya	160°C	1.0mm	52.8±2.8defg	0.6±1.6ab	24.6±2.7defghi
		1.5mm	57.3±3.9fghij	0.7±0.7ab	24.8±1.8efgh
		2.0mm	51.4±4.8bcdefg	10±1.2hijk	31.2±4.8hijklmnop
	170°C	1.0mm	54.6±2.5efgh	3.8±1.1cde	31.5±3.4ijklmno
		1.5mm	54.6±0.7fg	6.4±3.6cdefghi	32.9±3.2klmnop
		2.0mm	55.7±4.4efghi	2.6±0.6bc	31.1±4.4hijklmno
	180°C	1.0mm	50.6±5.5bcdefg	4.3±0.8de	28.7±1.8ghijk
		1.5mm	53.1±4.5defgh	8.5±1.5ghi	33.2±2.2lmno
		2.0mm	49.8±1.4cde	7.9±2.6fghij	32.2±2.7kmno

[1] Values are means of three determinations± standard deviation.

[2] Values with the same letters in the same column are not significantly different at 5% level of significance.

Table 5.3 Continued

Cultivar	Frying temperature	Slice Thickness	L	a*	b*
Sheherekea	160°C	1.0mm	51.9±1.3def	-0.6±0.3a	18.8±0.8ab
		1.5mm	48.8±1.5cd	11.1±3.1hiklm	29.1±2.8hijkl
		2.0mm	52.4±1.6def	5.3±3.7cdefgh	29.1±3.3ghijklm
	170°C	1.0mm	55.9±4.8efghi	0.8±1.4ab	26.6±1.2fghi
		1.5mm	50.5±3.3cdef	6.9±2.8defghi	30.3±0.9jkl
		2.0mm	52.1±4cdefg	9.6±3.8fghijklm	31.7±4.2iklmnop
	180°C	1.0mm	55±2.5efgh	3.7±0.5cd	31.2±1.1klm
		1.5mm	54.1±4.5defgh	6.3±3.3cdefghi	30.2±1jkl
		2.0mm	48.8±3bcde	7.5±2.1efghi	31.3±1.7kjklm
Tigoni	160°C	1.0mm	55.7±3.1efgh	2.1±2.2abcd	28.1±3.4ghijkl
		1.5mm	56.6±1.5gh	2.3±2.3ab	27.6±4.4efghijk
		2.0mm	50.8±5.1bcdefg	6±0.5ef	27.6±2.2ghijk
	170°C	1.0mm	55.3±0.7fg	-0.2±0.3a	21.4±1.1cd
		1.5mm	58.9±4.3fghijk	2.6±3.2abcdef	26.9±2.3fghij
		2.0mm	48±2.1bcd	4.6±2.1cdef	26.8±2.9fghijkl
	180°C	1.0mm	53.9±3.6defgh	4.5±0.6de	27.5±2.0ghij
		1.5mm	51±1.9de	5.3±2.3cdefg	27.7±2.7ghijk
		2.0mm	51.8±1.2de	6.2±1.6efg	29.4±1.3ijk

[1] Values are means of three determinations± standard deviation.

[2] Values with the same letters in the same column are not significantly different at 5% level of significance.

89 % of the potato crisps processed from the four potato varieties were light colored with L*>50. The lightest crisp was of slice thickness 2mm from Dutch Robjin fried at 180°C with a lightness level of 63.7. The least yellow was the crisp from Tigoni variety fried at 170°C and slice thickness of 2mm. The highest value of yellowness parameter was from the crisps produced from Dutch Robjin fried at 180°C with a slice thickness of 1.5mm. Most of the samples tended towards red as the redness parameter veered towards the positive indicating high degree of browning.

Crisps produced from the potatoes fried at 160°C at a slice thickness of 1.5mm from Sheherekea variety had the highest degree of redness of 11.1. This is an indication of excessive browning while those processed from the same variety and temperature but thickness of 1.0mm had the least browning due to the negative value -0.6. The positive value of the yellowness parameter b* is an indication that all the potato crisps in the processed tend towards yellow.

All the varieties were all light and no significance difference ($P \leq 0.05$) existed between them. The redness parameter in all varieties had positive values and in Sherehekea and Kenya Mpya which had the highest values of a* had no significant differences between them but the two differed significantly ($P > 0.05$) with Dutch and Tigoni. Kenya Mpya had the highest value of the yellowness parameter while Tigoni had the least and there was no significance difference among Dutch Robjin, Sheherekea and Kenya Mpya but the three significantly differed with Tigoni.

Temperature has a great influence on the CIE colour parameters with frying temperature of 170°C having the highest lightness parameter and no significance difference ($p > 0.05$) existing among the three frying temperatures. Temperatures of 180 C had the highest redness parameter while that of 160°C recording the lowest a* values. No significance difference ($p > 0.05$)existed in the redness parameters between the 160°C and 170°C frying temperatures but the two significantly differed with that at 180°C. The b* values differed significantly ($p \leq 0.05$) across the three frying temperatures with 180°C giving the highest and 160°C the lowest yellowness parameter values.

The thicker the potato crisp the less lighter it was since the slices of 2.0mm were least light. Between slice thickness 1.0mm and 1.5mm there was no significance difference ($p > 0.05$) but the two thicknesses significantly differed ($P \leq 0.05$) in the lightness parameter with the thickness of

2.0mm. The redness parameter and the yellowness parameter varied linearly with the thickness of the potato chips; the thicker the potato slices the higher the values of a* and b*. Least browning and least yellowing were detected in the potato crisps processed at 1.0mm while those of 2.0mm had higher average values of a* and b*. In the redness parameter, the values significantly differed ($P \leq 0.05$) among the three levels of thickness while in the yellowness parameter there was no significance difference in the between the thicknesses 2.0mm and 1.5mm but the two differed significantly with 1.0mm thicknesses.

Table 5.4 shows the levels of acrylamide in the crisps from the processed potato crisps from four Kenyan varieties.

Table 5.4: Levels of acrylamide in crisps from the four potato varieties grown in Kenya.

Variety	Frying temperature	Slice thickness	Acrylamide	Variety	Frying temperature	Slice thickness	Acrylamide
Dutch Robjin	160°C	1.0mm	3150±64ab	Sheherekea	160°C	1.0mm	3149±192ab
		1.5mm	4094±52cd			1.5mm	4073±937abcde
		2.0mm	5130±127ef			2.0mm	6051±323ghi
	170°C	1.0mm	3129.±434ab		170°C	1.0mm	5671±1200defghi
		1.5mm	3332±29b			1.5mm	5009±471def
		2.0mm	4813±67e			2.0mm	5599±803efgh
	180°C	1.0mm	4599±119de		180°C	1.0mm	7148±1521ghijklm
		1.5mm	4874±88e			1.5mm	7883±10l
		2.0mm	4917±199ef			2.0mm	9164±473mno
Kenya Mpya	160°C	1.0mm	3233±332ab	Tigoni	160°C	1.0mm	3702±489bcd
		1.5mm	5934±246gh			1.5mm	5209±399efg
		2.0mm	8783±860lmno			2.0mm	4987±93ef
	170°C	1.0mm	7048±1022hijkl		170°C	1.0mm	3776±74c
		1.5mm	11396±1091pqrst			1.5mm	4895±740defg
		2.0mm	12127.±1395qrstuv			2.0mm	5058±946defg
	180°C	1.0mm	13480±1030tuvwx		180°C	1.0mm	5933±555fgh
		1.5mm	12351±893rstuv			1.5mm	3627.±3.5c
		2.0mm	13150±1504stuvw			2.0mm	5412±529efg

[1] Values are means of six determinations± standard deviation.

[2] Values with the same letters in the same column are not significantly different at 5% level of significance.

Potato crisps from Kenya Mpya variety fried at 180°C and slice thickness 1.0mm recorded the highest levels of acrylamide (13480 ppb) with Dutch Robjin fried at 170°C and sliced at 1.0mm recording the lowest levels of the acrylamide (3129 ppb).

The acrylamide levels differed significantly (P≤0.05) across all the varieties with Kenya Mpya having the highest levels of acrylamide and the lowest levels being recorded in Dutch Robyjn. Temperature influences significantly acrylamide formation and the higher the temperature the higher the levels of acrylamide formed. Potato crisps fried at temperatures of 180°C recorded the highest levels of acrylamide followed by 170°C and the least levels were those processed at 160°C. The levels of acrylamide significantly varied (P≤0.05) across the three temperatures. The thicker the slice of the potato crisp the higher the degree of acrylamide formation as observed in the analysis carried out. Higher levels of acrylamide were formed at 2.0mm thickness and least levels at 1.0mm. The levels of acrylamide formed differed significantly (P≤0.05) at 95% confidence interval across the three thicknesses.

Table 5.5 indicates the Pearson correlation values between acrylamide content, percentage moisture content and L*, a* and b* colour parameters.

Table 5.5: Correlation between acrylamide content, moisture content and colour parameters for potato crisps processed from the four potato varieties.

Parameters	Moisture content	Acrylamide
Moisture content	1	0.287a
Acrylamide	0.287a	1
L	-0.156a	-0.184
a*	0.093a	0.467*
b*	0.0889a	0.527**

N=36; a not significant at p>0.05

* Significantly different at p≤0.05

**significant at p≤ 0.001

There was a weak positive relationship between moisture content and acrylamide (r=0.287).

A positive relationship existed between acrylamide and the redness (a*) (r=0.467) and yellowness (b*) (0.527) parameters. A very weak negative relationship existed between the both moisture content & acrylamide and the L* value of the colour parameter. No relationship existed between moisture and a* and b* colour parameters.

Table 5.6 indicates the correlation values between the sugars and acrylamide content.

Table 5.6: Correlation values between acrylamide content, reducing sugars and sucrose for potato crisps processed from the four potato varieties.

Parameters	Fructose	Glucose	Sucrose	Acrylamide
Frucose	1	0.834*	0.963**	0.444*
Glucose	0.834*	1	0.952*	0.762*
Sucrose	0.963**	0.952*	1	0.639**
Acrylamide	0.444*	0.762*	0.639**	1

N=4; * significantly different at$P\leq0.05$

**significant at $P\leq 0.001$

There was a positive correlation between glucose, fructose, sucrose and the acrylamide levels. The correlation between sucrose and the acrylamide levels was a weak positive relationship (r=0.444).

The correlation values among the reducing sugars and the acrylamide content showed a great significance difference ($P>0.05$). The correlation values between the acrylamide values and sucrose showed significant difference ($P\leq0.001$).

The results of sensory analysis for the potato crisps processed under different temperatures and slice thickness are given in Tables 5.7.

Table 5.7: Sensory attributes of the potato crisps processed from the four varieties.

Cultivar	Frying temperature	Slice thickness	Colour	Flavour	Texture	Oiliness	Overall acceptability
Dutch Robjin	160°C	1.0mm	3.2±1.2b	3.5±1.4c	3.6±1.5bc	3.2±1.2a	4.5±1.1cd
		1.5mm	4.1±1.4de	4.2±1.1de	4.6±1.0ef	3.9±1.4bc	4.5±0.7cd
		2.0mm	4±1.2d	3.9±1.7d	4.2±1.1de	3.6±1.3b	4.6±0.7d
	170°C	1.0mm	4.5±1.6d	4.4±1.3ef	4.4±1.3de	4.1±1.4cd	4.5±1.1cd
		1.5mm	3.9±1.2cd	2.7±1.2a	2.9±1.8a	3.9±1.4bc	3.5±1.2ab
		2.0mm	4.7±2.1de	3.7±1.8cd	4.3±1.6de	4.3±1.7cd	4.2±1.6c
	180°C	1.0mm	4.3±1.5de	3.8±1.5cd	4.7±1.8ef	4.4±1.4d	4.6±1.8cdef
		1.5mm	5±1.4fg	4.6±1.1ef	4.9±1.4fghi	5.1±1.7ef	5.1±1.5ef
		2.0mm	4.6±1.3e	5.3±0.8gh	5±1.2fg	5±1.3ef	5.4±1.2f
Kenya Mpya	160°C	1.0mm	3.8±1.3cd	4.1±1.6de	3.7±2.1c	3.8±1.5bc	3.6±1.3ab
		1.5mm	4.6±1.5e	4.8±1.7fg	4.9±1.1fghi	4.5±1.3de	4.7±0.9cde
		2.0mm	4.1±1.3de	4.4±1.0ef	4.2±1.4de	4.7±1.8de	4.6±1.3cdef
	170°C	1.0mm	3.6±1.6c	4.7±1.5f	4.6±1.4ef	3.5±1.5ab	3.6±1.5ab
		1.5mm	3.1±1.3ab	4.4±1.3ef	4.8±1.3ef	4.7±1.2de	4.6±0.8cde
		2.0mm	5.4±1.2g	4.8±1.1fg	5.1±1.1fg	5±1.2ef	5.5±1.3fg
	180°C	1.0mm	4.1±1.4de	5±1.0fg	3.5±1.6bc	3.3±1.5ab	4±1.4bc
		1.5mm	3.9±1.3cd	4.9±1.1fg	5.2±1.0fg	5.1±1.0ef	4.8±1.2de
		2.0mm	3.5±2.0bc	4.9±1.4fg	4.9±1.4fghi	4.8±1.2de	4.7±1.5cde

[1] Values are means of ten determinations ± standard deviation.

[2] Values with the same letters in the same column are not significantly different at 5% level of significance

[3] Evaluation was done on 7-point hedonic scale. A score of 4 was the acceptable lower limit.

Table 5.7 Continued

Cultivar	Frying temperature	Slice thickness	Colour	Flavour	Texture	Oiliness	Overall acceptability
Sheherekea	160°C	1.0mm	3.5±1.5bc	3.8±1.6cd	4.2±0.8de	3±1.6a	3.6±1.2ab
		1.5mm	4±1.4d	3.6±1.8cd	4.5±1.4e	4.4±1.7d	4.2±1.9c
		2.0mm	3.9±1.1cd	2.8±1.4ab	3.8±1.5c	3.3±1.3ab	3.5±1.3ab
	170°C	1.0mm	3.9±1.4cd	3.3±1.6bc	3±1.4ab	3.4±1.6ab	3.4±1.2a
		1.5mm	4.4±1.6e	4±1.9de	4.1±1.4d	3.8±1.5bc	4±1.5bc
		2.0mm	4.2±1.1de	3±1.9ab	3.5±1.4b	3.5±1.7ab	3.4±2.0a
	180°C	1.0mm	4.2±1.0de	3.4±1.7bc	3.7±1.6c	4.2±1.0cd	3.8±1.8b
		1.5mm	3.4±1.1bc	3.1±2.0b	3.1±1.7a	3.2±1.4a	3.6±1.9ab
		2.0mm	4.2±1.7de	4.3±1.3e	3.6±1.1bc	3.7±0.9bc	3.6±1.2ab
Tigoni	160°C	1.0mm	3.3±1.5bc	4.1±1.2de	3.9±1.5c	3.3±1.5ab	3.9±1.3bc
		1.5mm	4±1.5d	3.5±1.4c	3.6±1.3bc	3.2±1.4a	3.9±1.0bc
		2.0mm	4.1±1.6de	4±1.2de	4.8±1.7ef	3.9±1.2bc	4.5±1.4cd
	170°C	1.0mm	5.3±1.9g	4.1±2.0de	4.6±1.6ef	4.2±1.2cd	4.7±1.6cde
		1.5mm	4.8±1.6f	4.3±1.3e	4.6±1.6ef	4.4±1.5d	5±1.1de
		2.0mm	5±1.1fg	5.1±1.6g	5.5±1.0gh	4.6±1.2de	5.3±1.2ef
	180°C	1.0mm	4.2±1.0de	4.3±1.3e	4.7±2.0ef	5±1.2ef	4.8±1.1de
		1.5mm	4.6±1.3e	4.2±1.2de	4.2±1.0de	4.3±1.8cd	4.2±1.3c
		2.0mm	3.6±1.6c	3.6±1.6cd	4.2±1.6de	4.3±1.6cd	4±1.3bc

[1] Values are means of ten determinations± standard deviation.

[2] Values with the same letters in the same column are not significantly different at 5% level of significance

[3] Evaluation was done on 7-point hedonic scale. The acceptable lower limit was a score of 4.

The most acceptable crisps was that processed from Kenya Mpya 170°C at 2.0mm which had a score of 5.5 similarly was its colour also. The crisps with texture that was much preferred was from Tigoni processed at 170°C and thickness of 2.0mm with a score of 5.5 while the best flavour was from Dutch Robjin processed at 180°C and thickness of 2.0mm with a score of 5.3. the oiliness that scored highly i.e 5.1 was from Dutch Robyjn processed to 1.5mm and temperature of 170° C.

In sensory evaluation, Sheherekea had the lowest score (3.8) in preference by the panelists in colour. This can be attributed to the high reducing sugars that it possessed and also uneven browning on the surface. Kenya Mpya was the most preferred in terms of flavor with a score of 4.7 while Sheherekea was the least scored 3.5. In the overall acceptability there existed no significance difference between Dutch Robjin, Tigoni and Kenya Mpya but the three significantly differed with Sheherekea Dutch Robjin scored highly (4.5) followed while Sheherekea was the least accepted score of 3.9.

In the slice thickness, 2.0mm was highest in acceptability, texture and flavor with a score of slightly greater than 4 ($P \leq 0.05$). There was no significant difference $P > 0.05$ in colour in the three thicknesses as they 4.3, 4.1 and 4.1. In oiliness the 2.0mm scored poorly less than four and this can be related to the high moisture content thus a soggy product.

5.5.2 Discussion

5.5.2.1 Characteristics of potatoes used in crisps processing

The levels of dry matter content in the four varieties used in this study are within what was recommended (greater than 20%) for crisp production by Kabira and Lemaga (2003). The amount of dry matter content indicates the level of maturity of the tubers and the lower the dry matter content the lesser the physiological maturity of the tubers. Low dry matter content in potato tubers results from early tuber harvesting and this leads to accumulation of high levels of reducing sugars due to conversion of dry matter content of potatoes changes throughout growth similarly the ratios of sugars to starch. Typically, early harvested, 'new' potatoes have very thin skins, low dry matter percentages of about 16 % and high concentrations of reducing sugars compared with tubers harvested at maturity (Seal et al., 2008). Low dry matter content affects the general quality of the crisps as more heat will be needed to evaporate the water and will results in too oily and soggy crisps (Kabira and Lemaga 2003). While high dry matter content will result in too hard and dry crisps that is unacceptable. Thus it affects the texture and oiliness of the product which ultimately determines the overall acceptance of the product by the consumers (Kabira and Lemaga, 2006).

The colours of the crisps are influenced to a large extent by the reducing sugar of the tubers used in their production. Reaction between reducing sugars and the amino acids present in the tubers are responsible for the non enzymic browning (maillard reaction) of fried potato products that determines not only the colour but also the acrylamide content and finally the total acceptability of the potato crisps. High levels of reducing sugars greater than 0.05% on wet weight basis are not acceptable for the processing of crisps. The lower the levels of reducing sugars the lesser the browning of crisps after processing. Reducing sugar levels are influenced by maturity levels thus early harvesting of the tubers results in higher levels of reducing sugars as not enough reducing sugars have been converted to starch (Kumar et al., 2004). Storage also influences the levels of reducing sugars with reducing sugar levels accumulating to higher levels when stored at low temperatures (cold senescence) (Hertog et al., 1997). Starch levels are high when tubers are harvested at maturity and this inhibits browning since less maillard reactions take place. The levels of sucrose and the reducing sugars obtained were within the range of those reported by Seal et al. (2008). Higher levels of reducing sugars in Kenya Mpya resulted in darker crisps colour than in Tigoni. Sucrose concentrations were greater than the 0.15% suggested by Kumar et al. (2004) thus the possibility that the levels were higher at harvest and some were converted to the reducing sugars.

5.5.2.2. Potato crisps characteristics

5.5.2.2. 1 Moisture content of the processed potato crisps

The differences in moisture contents indicate that when frying not all the water evaporated from the potato crisps. In general these values were within the range reported by Abong et al., (2009) and are also less than the recommended 5% by the EAS (2010). The moisture content of the potato crisps is normally lowered to levels below 2·5 % resulting in core temperatures above 120°C. Therefore, acrylamide is formed through the whole product resulting in higher contents in crisps than in French fries despite shorter frying times (Seal et al., 2008).

The moisture content of the potato crisps influences the acceptability of the product and also the stability of the crisps. High moisture content results in low shelf life due to rancidity caused by lipid oxidation. Therefore lower moisture contents in this study indicate the crisps have lower shelf life as they are prone to rancidity.

5.5.2.2.3 Colour of the processed potato crisps

Colour of potato crisps is determined by the levels of reducing sugars and the amino acids whose interaction result in non enzymic browning. Higher levels of reducing sugars result in dark brown crisps as opposed to the golden brown colour. (Hamernik and Hanneman, 1998; Guar et al., 1999; Olsson et al., 2004; Abong' *et al*., 2009).

All the potato varieties used for the crisps production resulted in crisps of lightness parameter greater than 50. The redness parameters (a*) of the crisps were all positive indicating browning due to either excessive reducing sugars concentration with highest levels recorded in Sheherekea and Kenya Mpya which no significant (P>0.05) difference between the levels. These two varieties had the highest levels of reducing sugars as observed in figure two. The lowest value of the redness parameters was recorded in the Tigoni variety which had the lowest levels of reducing sugars. These findings are in tandem with the findings that the higher the reducing sugars the higher the non-enzymic browning due to maillard reaction which results in darker and bitter products which are unacceptable to the consumers.

There was no significant (P>0.05) difference in the lightness parameter (L*) of the potato crisps processed at the three temperatures; with the L* in all the temperatures being greater than 50 indicating that all the products were light. The higher the frying temperature the higher the browning indicated by the redness parameter of the potato crisps which was highest in those fried at 180°C and the lowest being those processed at 160°C. This indicates excessive browning due to the high processing temperatures.

The thicker the slice thickness the higher degree of browning as indicated by the higher values of the redness parameter (a*) since the amount of the precursors interacting are more than in the thin slices.

5.5.2.2. 4 Acrylamide levels in potato crisps

There was a significant ($P \leq 0.05$) difference in the levels of acrylamide in crisps from the four potato varieties. The potato variety influences the amount of acrylamide levels formed due to differences in reducing sugars, the dry matter content, cold-sweetening and senescence-sweetening were also found to be variety-dependent by Kumar et al., (2004). Kenya Mpya recorded the highest levels of acrylamide with Dutch Robjin recording the lowest levels of acrylamide formed. Acrylamide formation is influenced by the levels of reducing sugar in the potato tubers and since Kenya Mpya had the highest concentration of reducing sugars thus the high levels of acrylamide formed. Irreversible combination of reducing sugars and asparagine through the maillard reaction results in acrylamide formation in plant based food stuffs such as (Amrein et al., 2003). Reducing sugars are the limiting factor in acrylamide formation since the asparagines levels in raw potatoes only vary within a certain range, while the reducing sugars depends on variety, maturity, and storage among other growing and processing conditions(Olsson et al., 2004; De Wilde et al., 2005; Abong' et al., 2009).

The temperatures of processing the potato crisps were between 160°C and 180°C which had been found to be the optimal temperatures for acrylamide formation as reported by various authors thus the high levels (Mottram et al., 2002; Taubert *e*t al. 2004)). The levels of acrylamide in the current study are quite higher than those reported by EFSA (2005) or Codex (2003). This can be attributed to the high frying temperatures where there is optimal development of acrylamide. Also high reducing sugars and and other additives that contain reducing sugars increases the levels of acrylamide. Frying the potatoes at temperatures lower than 160°C reduces the concentration of acrylamide produced (Pedreschi et al., 2006), but quality characteristics of the product like texture, colour and oil content, are negatively affected and consequently the acceptability of the product by the consumer may be reduced. Though, temperatures higher than 200ºC or prolonged processing time may cause a decrease of acrylamide level due to intensified degradation reactions of acrylamide to glycidamide (Rydberg et al., 2003; Taubert et al., 2004).

There was significance in the levels of acrylamide depending on the thickness of the potato crisp. The thicker the slice the higher the acrylamide content this can be attributed to the higher concentrations of the reactants than the thinner slices (Pedreschi et al., 2005).

There was a strong positive relationship between fructose and sucrose with the acrylamide levels where r=0.762 for glucose and 0.639 for sucrose while there exists a moderate positive relationship between fructose and acrylamide r=0.442. This is in agreement with the findings that the concentration of reducing sugars determines the extent of formation of acrylamide as potatoes are generally high in the amino acid asparagine (Amrein et al., 2003). The concentration of sucrose in potatoes destined for processing into chips at harvest should be less than 1·5 mg/g to minimise accumulation of reducing sugars in long term storage at intermediate temperatures. Temperatures during production affects the levels of acrylamide formation as found out by Amrein et al., 2003). According to Biedermann et al., (2002) and Pollien et al., (2003) fructose was reported to be more efficient than glucose in forming acrylamide. It was also concluded that acrylamide is formed in comparable amounts with several mono- or disaccharides.

There exists a weak positive relationship between the moisture content and acrylamide formation. This is because the lower the moisture content the higher the dry matter and thus the reactants are brought close together and thus higher rate of acrylamide formation. With the decrease in the moisture, the formation of acrylamide was increased accordingly (Ye et al., 2011).

A positive correlation between acrylamide and the redness parameter a* r=0.332 is in agreement with those reported by various authors who correlated the colour of crisps as an indication of the acrylamide content (Pedreschi et al., 2005, Jackson & Al-Taher 2005; Viklund et al., 2007; Go¨kmen et al., 2007). This also confirms that acrylamide formation is a as a result of maillard reactions which determines browning (Isherwood., 1973 and Shallenberger et. al., 1959). There is a less close correlation between acrylamide and colour for large surface-to-volume material, such as potato crisps, in comparison with small surface-to-volume material, such as French fries as observed above (Taubert et al., 2004); thus the weak relationship between the redness parameter and the acrylamide content. In the sensory evaluation, the highest scores were just slightly above 5.0 indicating "like slightly." And thus no variety came out as the most liked since none scored above 7.0 (like very much). This can be attributed to the high frying temperatures and the reducing sugars which was quite high resulting in browning of the products. There is significance difference within each product range indicating difference in preference by the panelists. Kenya Mpya was the overall preferred in terms of colour and acceptability (>5). Thus processing of this variety at 170°C and slice thickness of 2.0mm is the most preferred for the crisps production. Dutch Robjin variety is the most used variety in the production of potato crisps in Kenya and this can be attributed to its desirable flavor and oiliness as earlier indicated. Low scores exhibited by other varieties and temperatures can be attributed to excessive browning and oiliness. Texture of the potato crisps is determined by the dry matter content of the tubers and can be hard or soggy (Abong et al., 2011). Kenya Mpya scored highly on the textural and oiliness acceptance due to its high dry matter content with Tigoni recording the least score.

Crisps processed at temperatures of 170°C scored highly in colour compared to the other temperatures. In texture and overall acceptability there was no significant difference at the three temperatures while flavor and oiliness processing at 170°C and 180°C showed no significant difference but differed greatly with processing at 160°C. These findings agree with those of (Rojo and Vincent., 2008; Abong et al., 2011). In addition colour is the most important parameter that determines the acceptability of not only the potato crisps but also most products (Pedreschi et al., 2007).

5.6 Conclusions and recommendations

Potato tubers for crisps processing should be harvested when mature as indicated by low levels of reducing sugars and high percentage dry matter content. Higher reducing sugars results in excessive browning of the potato crisps and thus the higher the redness parameter. Harvesting of potato tubers should be done at maturity when the levels of reducing sugars are at their lowest levels this will reduce the acrylamide levels.

There is a linear relationship between acrylamide levels and the levels of reducing sugars, the processing temperature and the slice thickness of the crisps. Slice thicknesses of 1.5mm and 2.0mm are mostly preferred in the processing of these potato crisps by consumers.

There is need to establish a lower processing temperature to limit the browning and acrylamide formation. More studies need to be done in order to determine which reduction strategies need to be employed while using these varieties.

CHAPTER SIX

GENERAL CONCLUSIONS AND RECOMMENDATIONS

6.1 General Conclusions

The potato crisps obtained from the roadside vendors (non-branded) contains higher levels of acrylamide as opposed to the branded crisps. The levels in both the branded and unbranded samples obtained from the Kenyan market were higher than those reported in the literatures. There is a weak positive relationship between acrylamide and the redness parameter (browning).

There could be a risk of exposure to acrylamide which varies from person to person depending on individual intake of a particular brand.

The high contamination levels of the potato crisps increases exposure to acrylamide by consumers in Nairobi, Kenya. The exposure is higher for consumers of non-branded crisps which subsequently lead to lower Margin of Exposure indicating that there could be a possibility of a risk of exposure by the consumers.

The potato varieties that were used in the study had high levels of reducing sugars and thus the crisps that were produced from them were high in acrylamide and browning. There is a linear relationship between acrylamide levels and the levels of reducing sugars, the processing temperature and the slice thickness of the crisps. Slice thicknesses of 1.5mm and 2.0mm are mostly preferred in the processing of these potato crisps by consumers.

6.2 Recommendations

The acrylamide levels in the flavoured samples could not be detected in this study thus need to look into factors that could have hindered its detection. The current study only focused on potato crisps consumers and did not consider acrylamide contribution from total diet and seasonal variation. It also did not include children who may be more exposed. Therefore, need to for more research in these areas.

Potato tubers for crisps processing should be harvested when mature as indicated by low levels of reducing sugars and high percentage dry matter content. Higher reducing sugars results in excessive browning of the potato crisps and thus the higher the redness parameter. Harvesting of potato tubers should be done at maturity when the levels of reducing sugars are at their lowest levels this will reduce the acrylamide levels. There is need to establish a lower processing temperature to limit the browning and acrylamide formation. More studies need to be done in order to determine which reduction strategies need to be employed for effective acrylamide reduction with respect to Kenyan potato varieties.

Ministry of Public Health and Kenya Bureau of Standards should take the lead in ensuring that the code of conduct to reduce acrylamide is adhered to. Regular monitoring of fried products and risk analysis should be carried out in the Kenyan markets and measures taken to assure safety of the consumer.

7.0. REFERENCES

Abong', G. O**.,** Okoth, M.W., Karuri, E.G., Kabira, J.N and Mathooko, F.M. (2009a). Levels of reducing sugars in eight Kenyan potato cultivars as influenced by stage of maturity and storage conditions. *Journal of Animal and Plant Sciences, 2* (2): 76 – 84.

Abong', G. O., Okoth, M.W., Karuri, E.G., Kabira, J.N and Mathooko, F.M. (2009b). Nutrient contents of raw and processed products from Kenyan potato cultivars. *Journal of Applied Biosciences, 16*: 877-886.

Abong', G. O**.,** Okoth, M.W., Imungi, J.K., and Kabira, J.N (2010). Consumption patterns, diversity and characteristics of potato crisps in Nairobi, Kenya. *Journal of Applied Biosciences, 32*: 1942-1955.

Abong, G. O., Okoth, M. W., Imungi, J. K., & Kabira, J. N. (2012). Effect of Slice Thickness and Frying Temperature on Color, Texture and Sensory Properties of Crisps made from Four Kenyan Potato Cultivars. *American Journal of Food Technology*, *6* (9): 753-762.

Abramsson-Zetterberg, L. (2003). The dose–response relationship at very low doses of acrylamide is linear in the flow cytometer-based mouse micronucleus assay. *Mutation Resistance, 535* (2): 215–222.

Ahn JS, Castle L., Clarke D.B., Lloyd A.S., Philo M.R., and Speck D.R (2002). *Food Additives & Contaminants, 19*: 1116–1124.

Amrein, T.M., Bachmann, S., Noti, A., Biedermann, M., Barbosa, M.F., Biedermann-Brem, S., Grob, K., Keiser, A., Realini, P., Escher, F., and Amado, R. (2003). Potential of acrylamide formation, sugars, and free asparagine in potatoes: A comparison of cultivars and farming systems. *Journal of Agricultural and Food Chemistry 51*: 5556–5560.

Amrein, T.M., Andres, L., Escher, F., and Amadò, R. (2007). Occurrence of acrylamide in selected foods and mitigation options. *Food Additives and Contaminants,* 24, (1): 13-25.

Annola, K., Karttunen, V., Keski-Rahkonen, P., Myllynen, P., Segerback, D., Heinonen, S., and Vahakangas, K.(2008). Transplacental transfer of acrylamide and glycidamide are comparable to that of antipyrine in perfused human placenta. *Toxicological Letters. 182*: 50–56.

Arisseto, A.P., Toledo, M.C., and Govaert, Y. (2007). Determination of acrylamide levels in selected foods in Brazil. *Journal of Food Additive and Contaminants, 24*: 236–241.

AOAC, (1980). Official methods of analysis, 13th ed., *Association of Official analytical chemists*, Washington, DC, USA.

Arribas-Lorenzo, G., and Morales, F.J. (2009). Dietary exposure to acrylamide from potato crisps to the Spanish population. *Food Additive and Contaminants*, *26*: 289–297

Baum, M., Fauth, E., Fritzen, S., Herrmann, A., Mertes, P., Merz, K., Rudolphi, M., Zankl, H., and Eisenbrand, G., (2005). Acrylamide and glycidamide: genotoxic effects in V79-cells and human blood. *Mutation Resistance, 580*: 61–69.

Becalski, A.; Lau, B. P.-Y.; Lewis, D.; Seaman, S. Acrylamide in Foods: Occurrence and Sources. *Abstracts of 116th Annual AOAC International Meeting*, Los Angeles, CA, Sept 22-26, 2002; AOAC: Gaithersburg, MD, 2002; pp 125-126.

Becalski, A., Lau, B.P., Lewis, D., and Seaman, S.W. (2003). Acrylamide in foods: Occurrence, sources, and modeling. *Journal of Agricultural and Food Chemistry. 51*: 802–808.

Becalski, A., Lau, B. P. Y., Lewis, D., Seaman, S. W., Hayward, S., Sahagian, Ramesh, M.,and Leclerc, Y. (2004). Acrylamide in French fries: Influence of free amino acids and sugars. *Journal of Agricultural and Food Chemistry, 52*: 3801–3806.

Besaratinia, A., and Pfeifer, G. P. (2003). Weak yet distinct mutagenicity of acrylamide in mammalian cells. *Journal of the National Cancer Institute*, *95*: 842-843.

Biedermann, M., Noti, A., Biedermann-Brem, S., Mozzetti, V., and Grob, K. 2002. Experiments on acrylamide formation and possibilities to decrease the potential of acrylamide formation in potatoes. *Mitteilungen aus Lebensmitteluntersuchung und Hygiene, 93*: 668–687.

Biedermann-Brem S, Noti A, Grob K, Imhof D, Bazzocco D, Pfefferle A (2003). *European Food Research and Technology,* 217: 369–373

Biedermann, M., Grundbock, F., Fiselier, K., Biedermann, S., Burgi, C., and Grob, K. (2010). Acrylamide monitoring in Switzerland, 2007–2009: results and conclusions. *Food Additives and Contaminants*. Part A *27:* 1352–1362.

Blasiak, J., Gloc, E., Wozniak, K., and Czechowska, A. (2004). Genotoxicity of acrylamide in human lymphocytes. *Chemico- Bioligical Interactions, 149*: 137–149.

Bongers, M.L, Hogervorst, J.G.F, Schouten, L.J., Goldbohm, R.A., Schouten, H.C., Goldbohm, R.A., and van den Brandt, P.A.. (2012). Dietary Acrylamide Intake and the Risk of Lymphatic Malignancies: The Netherlands Cohort Study on Diet and Cancer. *PLoS ONE 7(6),* e38016.

Boroushaki, M.T., Nikkhah, E., Kazemi, A., Oskooei, M., and Raters, M. (2010). Determination of acrylamide level in popular Iranian brands of potato and corn products. *Food and Chemical Toxicology, 4:*, 2581–2584.

Burley, V.J., Greenwood, D.C., Hepworth, S.J., Fraser, L.K., de Kok, T.M., van Breda, S.G., Kyrtopoulos, S.A., Botsivali, M., Kleinjans, J., McKinney, P.A., and Cade, J.E (2010). Dietary acrylamide intake and risk of breast cancer in the UK women's cohort. *British Journal on Cancer, 103*: 1749–1754.

CAC (Codex Alimentarius Commission) (2009). Code of practice for the reduction of acrylamide in foods; CAC/RCP 67.

Castle L., and Eriksson, S. (2005). *Journal of AOAC International, 88:* 274–284.

Chakrabarti T., Ungeheuer P., 2002. Health implications of acrylamide in food, Food safety. Report of a Joint FAO/WHO Consultation. FAO, Geneva, 1-34.

Chen, F., Yuan, Y., Lui, J., Zhao, G., & Hu, X. (2008). Survey of acrylamide levels in Chinese foods. *Food Additives and Contaminants, Part B, 1:* 85–92.

Chiou, A., Kalogeropoulos, N., Boskou, G., & Salta, F.N. (2012).Migration of health promoting microconstituents from frying vegetable oils to French fries. *Food Chemistry, 133*: 1255–1263.

Ciesarova, Z., Kiss, E., and Kolek, E. (2006). Study of factors affecting acrylamide levels in model systems. *Czech Journal of Food Science, 24:* 133-137.

Claeys, W., Baert, K., Mestdagh, F., Vercammen, J., Daenens, P., De Meulenaer, B. Maghuin-Rogister G., and Huyghebaert, A. (2010). Assessment of the acrylamide intake of the Belgian population and the effect of mitigation strategies. *Food Additives and Contaminants, 27*(9): 1199–1207.

Confederation of the Food and Drink Industries of the EU (CIAA) (2006) The CIAA Acrylamide "Toolbox", pp. 1–35. Brussels: CIAA AISBL.

Council Directive Regulation (EC) 1272/2008 EC of 16 December 2008 on the classification, labelling and packaging of substances and mixture.

Croft, M., Tong, P., Fuentes, D., and Hambridge, T. (2004). Australian survey of acrylamide in carbohydrate-based foods. *Journal of Food Additives and Contaminants, 21*: 721–736.

Cummins, E., Butler, F., Gormley, R., and Brunton N. (2008). A methodology for evaluating the formation and human exposure to acrylamide through fried potato crisps. *Lebensmittel-Wissenschaft and Technologie, 41*: 854–867.

De Meulenaer, B., De Wilde, T., Mestdagh, F., Govaert, Y., Ooghe, W. Fraselle, S., Demeulemeester, K., Van Peteghem, C., Calus, A., Degroodt, J-M., and Verhe, R. (2008).

Comparison of potato varieties between seasons and their potential for acrylamide formation. *Journal of the Science of Food and Agriculture, 88*: 313–318.

De Wilde, T, De Meulenaer, B, Mestdagh, F, Govaert, Y, Vandeburie, S, Ooghe, W, Fraselle, S, Demeulemeester, K, Van, PC, Calus, A, Degroodt, JM & Verhe, R (2005) Influence of storage practices on acrylamide formation during potato frying. *Journal of Agricultural & Food Chemistry, 53:* 6550–6557.

Doerge, D.R., Gamboa da Costa, G., McDaniel, L.P., Churchwell, M.I., Twaddle, N.C., and Beland, F.A. (2005). DNA adducts derived from administration of acrylamide and glycidamide to mice and rats. *Mutation Resistance, 580*: 131–141.

Dybing, E., Farmer, P. B., Andersen, M., Fennell, T. R., Lalljie, S. P., Muller, D.J., Olin, S., Petersen, B.J., Schlatter, J., Scholz, G., Scimeca, J.A., Slimani, N., and Tornqvist, M., Tuijtelaars, S., and Verger, P. (2005). Human exposure and internal dose assessments of acrylamide in food*.* *Food Chemical Toxicology, 43*: 365–410.

East African Standards (EAS) (2010). Potato crisps-Specifications. East African Standards 745:2010.

European Food Safety Authority (EFSA) (2005). Opinion of the Scientific Committee on a request from EFSA related to a harmonized approach for risk assessment of substances which are both genotoxic and carcinogenic (Request No. EFSA-Q-2004-020). *European Food Safety Authority Journal, 280*: 1–31.

European Food Safety Authority (EFSA) (2011).

European Commission (EC). (2010) Indicative acrylamide values based on the EFSA monitoring data from 2007 – 2008. Commission recommendation, Brussels.

Fan, X., and Mastovska, K. (2006). Effectiveness of Ionizing Radiation in Reducing Furan andAcrylamide Levels in Foods. *Journal of Agricultural and Food Chemistry, 54*: 8266-8270.

FAO/WHO (2002) Health implication of acrylamide in food. Report of a Joint FAO/WHO Consultation. Geneva, Switzerland: World Health Organization.

Fischer, A., and Laing, J. (1991). Handbook for Primary Planning Operation Research Design, the Population Council, New York. Pp. 32-34.

Fiselier, K., Bazzocco, D., Gamma-Baumgartner, F., and Grob, K, (2006). Influence of the frying temperature on acrylamide formation in French fries. *European Food Research and Technology, 222,* 414–419.

Food and Drugs Authority (FDA). (2011). FDA survey on acrylamide in food: individual food products. (Accessed on 11th December 2012).

Food Drink Europe (FDE) (2011). Acrylamide toolbox accessed on 14th December, 2012 from
http://ec.europa.eu/food/food/chemicalsafety/contaminants/ciaa_acrylamide_toolbox09.pdf.

Friedman, M., Dulak, L., and Stedham ,M. (1995). A lifetime oncogenicity study in rats with acrylamide. *Fundamentals of Applied Toxicology*, *27*: 95-105.

Friedman, M. (2003). Chemistry, biochemistry, and safety of acrylamide: A review. *Journal of Agricultural and Food Chemistry, 51,* 4504-4526.

Foot RJ, Haase NU, Grob K., and Gondé P, 2007. Acrylamide in fried and roasted potato products: A review on progress in mitigation. *Food Additives & Contaminants, 24*, (1): 37-46.

Gamboa da Costa, G., Churchwell, M.I., Hamilton, L.P., Beland, F.A., Marques, M.M., and Doerge, D.R. (2003). DNA adduct formation from acrylamide via conversion to glycidamide in adult and neonatal mice. *Chemical Research in Toxicology, 16*, 1328–1337.

Ghanayem, B.I., Witt, K.L., El-Hadri, L., Hoffler, U., Kissling, G.E., Shelby, M.D. and Bishop, J.B. (2005). Comparison of germ cell mutagenicity in male CYP2E1-null and wildtype mice treated with acrylamide: evidence supporting a glycidamide mediated effect. *Biology of Reproduction, 72*, 157–163

Go¨kmen V., Senyuva H.Z., Dulek B., and Cetin A. E. (2007). *Food Chemistry, 101*:791–798

Grob, K. (2005) Reduction of exposure to acrylamide: achievements, potential of optimization, and problems encountered from the perspectives of a Swiss enforcement laboratory. *Journal of AOAC International, 88,* 253–261.

Girma, K.B., Lorenz, V., Blaurock, S., and Edelmann, F.T. (2005) Coordination chemistry of acrylamide. Coordination Chemistry Reviews, 249, (11–12), 1283–1293.

Guar, P.C., Singh, B., Pandey, S.K., Marwaha R.S., and Kumar, D. (1999). A new high dry matter potato variety for chipping. *Current Science, 79*, 722-724.

Guthrie, J.F., Lin, B.H., and Frazao, E. (2002) Role of food prepared away from home in the American diet, 1977 –78 versus 1994–96: Changes and consequences. *Journal of Nutrition Education and Behavior, 34*, 140–150.

Hamernik, A.J., and Hanneman, Jr. R.E. (1998). Breeding haploid species potato that chip from cold storage. *American Journal of Potato Research*, *75* (6): 278-279.

Haase, N. U. & Weber, L. (2003). Variability of sugar content in potato varieties suitable for processing. *Food Agriculture and Environment, 1*(3/4): 80–81.

Haase, N. U., Matthaus, B., and Vosmann, K. (2004). Aspects of acrylamide formation in potato crisps. *Journal of Applied Botany and Food Quality, 78*, 144–147.

Halford, N.G., Curtis, T.Y., Muttucumaru, N., Postles, J., Elmore, S. J., and Mottram, D. S. (2012). The acrylamide problem: a plant and agronomic science issue. *Journal of Experimental Botany, 63* (8), 2841-2851.

Henry, CJ, Lightowler, HJ, Strik, CM & Storey, M (2005) Glycaemic index values for commercially available potatoes in Great Britain. *British Journal of Nutrition, 94,* 917–921.

Hertog, MLATM, Putz, B., and Tijskens, LMM (1997).The effect of harvest time on the accumulation of reducing sugars during storage of potato (*Solanum tuberosum* L.) tubers: experimental data described, using a physiological based, mathematical model. *Potato Research, 40*, 69–78.

Heudorf, U., Hartmann, E., and Angerer, J. (2009). Acrylamide in children e exposure assessment via urinary acrylamide metabolites as biomarkers. International *Journal of Hygiene and Environmental Health, 212*, 135-141.

Hogervost, J. G. F., Schouten, L. J., Konings, E. J. M., Goldbohm, R. A., and van den Brandt, P. A. (2007). A prospective study of dietary acrylamide intake and the risk of endometrial, ovarian, and breast cancer. *Cancer Epidemiology, Biomarkers Prevention, 16***,** 2304–2311.

International Agency for Research on Cancer (1994). Monographs on the evaluation of carcinogenic risks to humans, some industrial chemicals. *International Agency for Research on Cancer, 60*, 389–433.

Isherwood F. A. (1973). Starch-sugar inter-conversion in solanum tuberosum . *Phytochemistry, 12*, 2579-2591.

Jackson, L. S., and Al-Taher F. (2005). Effects of consumer food preparation on acrylamide formation. In: Friedman M, Mottram D (eds) Chemistry and safety of acrylamide in food. *Springer, New York, pp 447–465*

Joint Expert Committee on Food Additives (JECFA). (2005). FAO/WHO, Joint FAO/WHO Expert Committee on Food Additives: Summary and Conclusions Report from Sixty fourth

Meeting, Rome, 8–17 February 2005. http://www.who.int/foodsafety/chem/jecfa/summaries/summary_report_64_final.pdf. (Accessed on 13th December, 2012)

Joint Expert Committee on Food Additives (JECFA) (2011). Evaluation of certain food additives and contaminants. 72nd report of the joint FAO/WHO expert committee on food additive. WHO Technical Report Series 959.

Johnson, K.A., Gorzinski, S.J., Bodner, K.M., Campbell, R.A., Wolf, C.H., Friedman, M.A., and Mast, R.W (1986). Chronic toxicity and oncogenicity study on Acrylamide incorporated in the drinking water of Fischer 344 rats. *Toxicoogy and. Applied Pharmacology*, *85*, 154–168.

Katz, J.M., Winter, C.K., Buttrey, S.E., and Fadel, J.G. (2012). Comparison of acrylamide intake from Western and guideline based diets using probabilistic techniques and linear programming. *Food and Chemical Toxicology, 50*, 877–883.

Kabira, J.N., and Lemaga, B. (2006). Quality Evaluation procedures for Research and food Industries Applicable in East and Central Africa. A publication of Kenya Agricultural Research Institute, Kenya.

Khalil, F.A., and Aziem, B.A. (2005). Effect of dietary acrylamide formed in potato crisps and toasted bread on rats. *Egyptian Journal of Natural Toxins, 2*, 57-70.

Kita, A., Brathen, E., Knutsen, S.H., and Wicklund, T. (2004). Effective ways of decreasing acrylamide content in potato crisps during processing. *Journal of Agricultural and Food Chemistry, 52*, 7011–7016.

Knol, J.J., Vicklund G., Linssen, J.P.H., Sjoholm, I.M., Skog, K.I. and Boekel, M.A.J.S. (2009). Kinetic modelling: A tool to predict the formation of acrylamide in potato crisps *Food Chemistry, 113, (1),* 103-109

Konings, E. J. M., Baars, A. J., van Klaveren, J. D., Spanjer, M. C., Rensen, P. M., and Hiemstra, M., (2003). Acrylamide exposure from foods of the Dutch population and an assessment of the consequent risks. *Journal of Food Chemistry & Toxicology, 41*, 1569–1579.

Kulkarni, K.D. and Govinden, N., (1994). Crisp Quality of Two Potato Varieties: Effects of Dehydration and Rehydration. *Journal of Science and Food Agriculture, 64*: 205- 210.

Kumar, D, Singh, B. P., and Kimar, P (2004) An overview of factors affecting sugar content of potatoes. *Annals of Applied Biology, 145*, 247–256.

Lachat, C., Van Camp, J., De Henauw, S., and Matthys, C. (2005). A concise overview of national nutrition action plans in the European Union Member States. *Public Health Nutrition, 8,* 266–274.

Larmond, E. (1977). Methods for sensory evaluation of food**.** Food Research Institute, Central Experiment Farm, Canada Dept. of Agriculture, Ottawa.

Larsson, S.C., Akesson, A., and Wolk, A. (2009). Long-term dietary acrylamide intake and breast cancer risk in a prospective cohort of Swedish women. *American Journal of Epidemiology, 169, (3),* 376–381.

Lin, Y., Lagergren, J., and Lu, Y (2011). Dietary acrylamide intake and risk of esophageal cancer in a population-based case-control study in Sweden. *International Journal of Cancer, 128,* 676–681.

Lipworth, L., Sonderman, J.S., Tarone, R.E., and McLaughlin, J.K. (2012). Review of epidemiologic studies of dietary acrylamide intake and the risk of cancer. *European Journal of Cancer Prevention, 21*, 375–386.

LoPachin, R. M. (2004). The changing view of acrylamide neurotoxicity. *Neuro Toxicology*, *25*, 617-630.

Maillot, M., Vieux, F., Amiot, M.J., and Darmon, N. (2010). Individual diet modeling translates nutrient recommendations into realistic and individual-specific food choices. *American Journal of Clinical Nutrition, 91*, 421–430.

Matthäus B, Haase N.U., and Vosmann K. (2004). Factors affecting the concentration of acrylamide during deep-fat frying of potatoes. *European Journal of Lipid Science Technology, 106,* 793-801.

Medeiros,R.V., Mestdagh,F., , De Muer, N., Peteghem, C., and De Meulenaer, B. (2012). Effective quality control of incoming potatoes as an acrylamide mitigation strategy for the French fries industry. *Food Additives and Contaminants, 27,* (4), 417–425.

Mestdagh, F., Lachat, C., Katleen Baert, K., Moons, E., Kolsteren, P., Van Peteghem, C., and De Meulenaer, B. (2007). Importance of a canteen lunch on the dietary intake of acrylamide. *Moecular Nutrition and Food Research, 51*, 509 – 516.

Mojska, H, Gielecin´ska, I., and Stos, K. (2012). Determination of acrylamide level in commercial baby foods and an assessment of infant dietary exposure. *Food and Chemical Toxicology, 50*, 2722–2728.

Mottram, D., Wedzicha, B., and Dodson, A. (2002). Acrylamide is formed in the Maillard reaction. *Nature, 419*, 448–449.

Matissek, R. (2009). Verbraucherinformationen zur Thematik Acrylamid bei Kartoffelchips (stand 04/09). http://www.lci-koeln.de/media/0904/Acrylamid_Matissek.pdf.

Ministry of Agriculture (MoA). (2005). Potato Standards In: The Crop Production and Livestock Act. Kenya Gazette Supplement no.38, Nairobi.

Ministry of Agriculture (MoA). (2007). Challenges in potato research In: The National Policy on Potato Industry presentation during the potato stakeholders meeting at KARI headquarters, Nairobi, Kenya.

Ministry of Agriculture (MoA). (2009). Mainstreaming the potato crop from orphan crop status. Proceedings of Round Table Africa (RTA) potato stakeholders' workshop held on 8th May 2009, Nairobi, Kenya.

Mottram, D. S., Wedzicha, B. L., and Dodson, A. T. (2002). "Acrylamide is formed in the Maillard reaction". *Nature 419,* (6906), 448–449.

Mottram, D. S. Low, M. Y., Koutsidis, G., Parker, J. K., Elmore, J. S. and Dodson, A. T. (2006). Effect of citric acid and glycine addition on acrylamide and flavor in a potato model system. *Journal of Agriculture and Food Chemistry, 54*, 5976–5983.

Nemoto, S., Takatsuki, S., Sasaki, K., and Maitani, T. (2002). Determination of acrylamide in food by GC/MS using 13C-labelled acrylamide as internal standard. *Journal of Food Hygiene Japan, 43*, 371–376.

O¨lmez H., Tuncay, F., O¨zcan N., and Demirel S. (2008). A survey of acrylamide levels in foods from the Turkish market. *Journal of Food Composition and Analysis, 2,* 564– 568.

Office of Environmental Health Hazard Assessment (OEHHA), 2012. Proposition 65. Available from, http://oehha.ca.gov/prop65/acrylamide.html, (Accessed on 11th December 2012).

Olsson, K., Svensson, R., and Roslund, C.-A. (2004). Tuber components affecting acrylamide formation and colour in fried potato: Variation by variety, year, storage temperature, and storage length. *Journal of Science Food & Agriculture, 84*, 447–458.

Ono, H., Chuda, Y., and Ohnishi-Kameyama, M. (2003). Analysis of acrylamide by LC-MS/MS and GC-MS in processed Japanese foods. *Journal of Food Additives and Contaminants, 20*, 215–220.

Pedersen, G.S., Hogervorst, J.G., Schouten, L.J., Konings, E.J., Goldbohm, R.A., and van den Brandt, P.A. (2010). Dietary acrylamide intake and estrogen and progesterone receptor-defined postmenopausal breast cancer risk. *Breast Cancer Research and Treatment, 122, (1),* 199–210.

Pedreschi, F., Kaack, K., and Granby, K. (2004). Reduction of acrylamide formation in potato slices during frying. *Lebensmittel-Wissenschaft and Technologie, 37*, 679–685.

Pedreschi, F., & Moyano, P. (2005). Oil uptake and texture development in fried potato slices. *Journal of Food Engineering, 70,* 557-563.

Pedreschi F., Moyano, P., Santis, N. and Pedreschi, R. (2007). Physical properties of pre-treated potato chips. *Journal of Food Engineering, 79, (4),* 1474–1482.

Pedreschi, P., Granby, B., and Risum, J. (2010a). Acrylamide Mitigation in Potato Chips by Using NaCl. *Food Bioprocess Technolology, 3*, 917–921.

Pedreschi, F., Segtnan, V.H., and S.H. (2010b). KnutsenOn-line monitoring of fat, dry matter and acrylamide contents in potato chips using near infrared interactance and visual reflectance imaging. *Food Chemistry, 121*, 616–620.

Pelucchi, CC., La Vecchia, C., Bosetti, C., Boyle, P., and Boffetta, P. (2011). Exposure to acrylamide and human cancer – a review and meta-analysis of epidemiologic studies. *Annals of Oncology, 22,* 1487–1499.

Peksa, A., Gołubowska, G., Aniołowski, K., Lisin´ska, G., and Rytel E. (2006). Changes of glycoalkaloids and nitrate contents in potatoes during chip processing. *Food Chemistry, 97*(1), 151-156.

Putz, B. (2004). Systematic studies on process optimization to minimize acrylamide. *Kartoffelbau, 55*, 188–192.

Pollien, P., Lindinger, C., Yeretzian C., and Blank, I. (2003). Proton transfer reaction mass spectrometry is a suitable tool for on-line monitoring of acrylamide in food and Maillard systems. *Analytical Chemistry*, *75*, 5488–5494.

Reynolds S., and Weintraub L. (1959). "Acrylamide Gel as a Supporting Medium for Zone Electrophoresis". *Journal of Science, 130*, (3377): 711.

Reynolds T., (2002). Acrylamide and Cancer: Tunnel Leak in Sweden Prompted Studies *Journal of National Cancer Institute, 94 (12), 876-878.*

Rice, J.M. (2005). The carcinogenicity of acrylamide*. Mutation Resistance, 580,* 3–20.

Rojo F. J and Vincent J. F. V. (2008). Fracture properties of potato crisps. *International Journal of Food Science & Technology, 43,*752–760.

Rosen, J., and Hellenas, K. (2002) Analysis of acrylamide in cooked foods by liquid chromatography tandem mass. *The Analyst, 127*, 880-882.

Rydberg, P., ErikssoN, S., Tareke, E., Karlsson, P., Ehrenberg, L., and Törnqvist, M. (2003). Investigation of factors that influence the acrylamide content of heated foodstuffs. *Journal of Agricultural and. Food Chemistry, 51*, 7012–7018.

Seal, C. J., De Mul, A., Eisenbrand, G., Haverkort, A. J., Franke, K., Lalljie S.P. D.,, and others (2008). Risk-Benefit Considerations of Mitigation Measures on Acrylamide Content of Foods – A Case Study on Potatoes, Cereals and Coffee. *British Journal of Nutrition. 99*, S1-S46

Schettgen, T., Broding, H. C, Angerer, J., and Drexler, H. (2002). Hemoglobin adducts of ethylene oxide, propylene oxide, acrylonitrile and acrylamide-biomarkers in occupational and environmental medicine. *Toxicology Letters, 134*, 65 -70.

Shallenberger, R.S., Smith, O., and Treadway, R.H., (1959). Role of sugars in the browning in potato chips. *Journal of Agricultural and. Food Chemistry, 7*, 274-279.

Sirot, V., Hommet, F., Alexandra Tard, A., and Leblanc. J.C. (2012). Dietary acrylamide exposure of the French population: Results of the second French Total Diet Study. *Food and Chemical Toxicology, 50*, 889–894.

Slayne, M. A., and Lineback, D. R. (2005). Acrylamide: Considerations for risk management. *Journal Association of Official Agricultural Chemists International, 88*, 227 –233.

Spivey, A. (2010). A matter of degrees: Advancing our understanding of acrylamide. *Environmental Health Perspectives, 118, (4),* A160–A167.

Stadler, R. H. and Scholz, G. (2004) Acrylamide: An update on current knowledge in analysis, levels in food, mechanisms of formation, and potential strategies of control. *Nutrition Reviews, 62,* 449–467.

Stadler, R. H., Robert, F., Riediker, S., Varga, N., Davidek, T., and Devaud, S. (2004). In-depth mechanistic study on the formation of acrylamide and other vinylogous compounds by the Maillard reaction. *Journal of Agricultural and Food Chemistry, 52*, 5550–5558.

Swedish National Food Administration, (2002). Swedish National Food Administration: Analysis of Acrylamide in Food (http://www.slv.se/acrylamide). Accessed on 4th April, 2013.

Taeymans D., Wood J., Ashby P., Blank I., Studer A., Stadler H S., and Gonde P. (2004): A Review of Acrylamide: An Industry Perspective on Research, Analysis, Formation, and Control. *Critical Reviews in Food Science and Nutrition*, *44* (5), 323-347.

Tareke, E., Rydberg, P., Karlsson, P., Eriksson, S., and Törnqvist. M. (2000). Acrylamide: A cooking carcinogen? *Journal of Chemical Research in Toxicology, 13*, 517–522.

Tareke, E., Rydberg, P., Karlsson, P., Eriksson, S., and Törnqvist, M. (2002). Analysis of acrylamide, a carcinogen formed in heated foodstuffs. *Journal of Agricultural and Food Chemistry, 50,* 4998-5006.

Tardiff R.G., Gargas M.L., Kirman C.R., Carson M.L. & Sweeney L.M. (2010). Estimation of safe dietary intake levels of acrylamide for humans. *Food and Chemical Toxicology, 48,* 658–667.

Tateo, F.,Bononi, M., and Gallone, F. (2010). Acrylamide content in potato chips on the Italian market determined by liquid chromatography tandem mass spectrometry International Journal of *Food Science and Technology*, *45*, 629–634.

Taubert, D., Harlfinger, S., Henkes, L., Berkels, R., and Schömig, E. (2004). Influence of processing parameters on acrylamide formation during frying of potatoes. Journal of *Agricultural and Food Chemistry 52,* 2735–2739.

U.S. Food & Drug Administration method *Detection and Quantitation of Acrylamide in Foods* dated June 20, 2002. Accessed on 25th of August, 2012 from http://www.perkinelmer.com/CMSResources/Images/4474337FAR_GCAcrylamideAnalysis.pdf

Viklund, G. A. I., Olsson, K. M., Sjo¨holm, I. M., and Skog, K.I. (2008). Variety and storage conditions affect the precursor content and amount of acrylamide in potato crisps. *Journal of Science, Food and Agriculture, 88*, 305–312.

Viklund, G. A. I., Olsson, K. M., Sjo¨holm, I. M., and Skog, K.I. (2010). Acrylamide in crisps: effect of blanching studied on long-term stored potato clones. *Journal of Food Composition & Analysis, 23* (2), 194-198.

Walingo, A., Lung'aho, C., N'gang'a, N., Kinyae, P.M., and Kabira, J.N. (2004). Potato marketing, storage, processing and utilization in Kenya. Proceedings of 6th Triennial congress of the African potato Association, Agadir, Morocco 5-10 April.

Weisshaar, R., and Gutsche, G. (2002). Formation of acrylamide in heated potato products-model experiments pointing to asparagine as precursor. *Deutsche Lebensmittel- Rundschau*, *98*, 397–400.

Wenzl, T., de la Calle, B., and Anklam, E. (2003). Analytical methods for the determination of acrylamide in food products: A review. *Food Additive and Contaminants*, *20*, 885–902.

WHO (2002). FAO/WHO Consultation on the Health Implications of Acrylamide in Food. Summary Report of a meeting held in Geneva, 25-27 June 2002.

Williams JSE, (2005). Influence of variety and processing conditions on acrylamide levels in fried potato crisps. *Food Chemistry, 90, 875-881*.

Wilson, K.M., Mucci, L.A., Cho, E., Hunter, D.J., Chen, W.Y., and Willett, W.C. (2009). Dietary acrylamide intake and risk of premenopausal breast cancer. *American Journal of Epidemiology, 169,* 954–961.

Woolfe, J.A. (1987). The potato in the human diet. Cambridge University Press, Cambridge, UK. Pp. 19-54.

Yaylayan, V.A., Wnorowski, A., and Locas, C.P. (2003). Why asparagine needs carbohydrates to generate acrylamide. *Journal of Agriculture and Food Chemistry*, *51,* 1753–1757.

Ye. H., Miao. Y.,, Zhao., C., and Yuan Y. (2011). Acrylamide and methylglyoxal formation in potato chips by microwaving and frying heating. *International Journal of Food Science & Technology*, *46* (9), 1921–1926.

Zhang, Y., Zhang, G., and Zhang, Y. (2005). Journal of Chromatography A, 1075, 1–21

Zhiqiang, E. L., and Scanlon, M. G. (2007). Modeling the effect of blanching conditions on the texture of potato strips. *Journal of Food Engineering, 81*(2), 292–297.

Zyzak, D., Sanders, R.A., Stojanovic. M., Tallmadge, D., Eberhart, B.L., Ewald, D.K., Gruber, D.C., Morsch, T.R., Strothers, M.A., Rizzi, G.P., and Villagran, M.D. (2003). Acrylamide formation in heated foods. *Journal of Agriculture and Food Chemistry*, *51*, 4782–4787.

ANNEX

Annex 1: Survey on consumption of potato crisps, Nairobi, Kenya

The aim of this study is to measure the current consumption pattern of potato crisps so as to form a basis for future monitoring and evaluation of the potato industry progress in Kenya. Your honest responses will be used to inform research team of the current situation, and will therefore be treated with utmost confidentiality; and will not be used for purposes other than research only. Your cooperation and participation is highly appreciated. Thank you for accepting to take part in this study.

Enumerator's name __________________ Start time___________ End time _________

Background information

1: General information

Classifying information	
Date of interview	
Location of interview	
Name of the outlet/building	
GPS coordinates of residence	
Date checked	
Date of data entry	

2: Respondent and general household information

2.1. Name of respondent	
2.2. Respondent gender	1= Male [ ], 2 = Female [ ]
2.3. Educational level	1=No formal education, 2=Primary, 3=Secondary (ordinary level), 4=Secondary(advanced level) 5=Tertiary [ ]
2.4. Age (years)	
2.5. Estimated body weight (kg)	
2.6. Estimated height (metres)	
2.7. Marital status	1=Married living with spouse, 2=Married but spouse away, 3=Divorced/separated, 4=Widow/widower,5=Not married, 6=Other (specify) ____________________________ [ ]
2.9. Main occupation	1=None, 2=Farming, 3=Salaried employment,4=Self-employed, 5 =casual worker, 6= student, 7=Other (Specify)_______

3. Consumer Study

3.1. Do you consume potato crisps? 1=Yes 2= No [ ]

3.2. If no above why? ____

3.3. If yes, how often do you consume crisps per week? Once daily [ ], twice daily [ ], Once per week [ ], twice a week [ ], thrice a week [ ], other (specify)____________

3.4. What unit package do you purchase at any given time you buy crisps? Less than 20g [ ], 30g [ ], 50g [ ], 70g [ ], other (specify) ________. State the price KSh_________

3.5. Do you consume all crisps you buy alone? Yes [ ], No [ ].

3.6. If no how much of the crisps you buy do you eat? ________. Who else consumes the crisps? ___________

3.7. Where do you usually purchase your crisps? Kiosks [ ], supermarkets [ ], street [ ], other (specify)______

3.8. Why do you buy from your preferred Retailer?

3.9. Quantity [ ], high quality [ ], Affordable [ ], Proximity [ ], Other [ ] (specify)__________________

4.0. Which brands of crisps do you often buy? What reasons?

Brand	Flavours				Reasons
	Onion	chilly	garlic	others	
1.					
2.					
3.					
4.					

4.1. What complaints, if any, have you ever raised on the crisps you buy? List in order of common complaints

1) ____
2) ____
3) ____

4.2. Can you describe your trend of crisps consumption in the last 2 years? Give reasons.

	Trend (increasing/decreasing)	Reasons
Consumption		

4.3. Any comments on potato crisps you would wish to share

____
____
____
____

Thanks