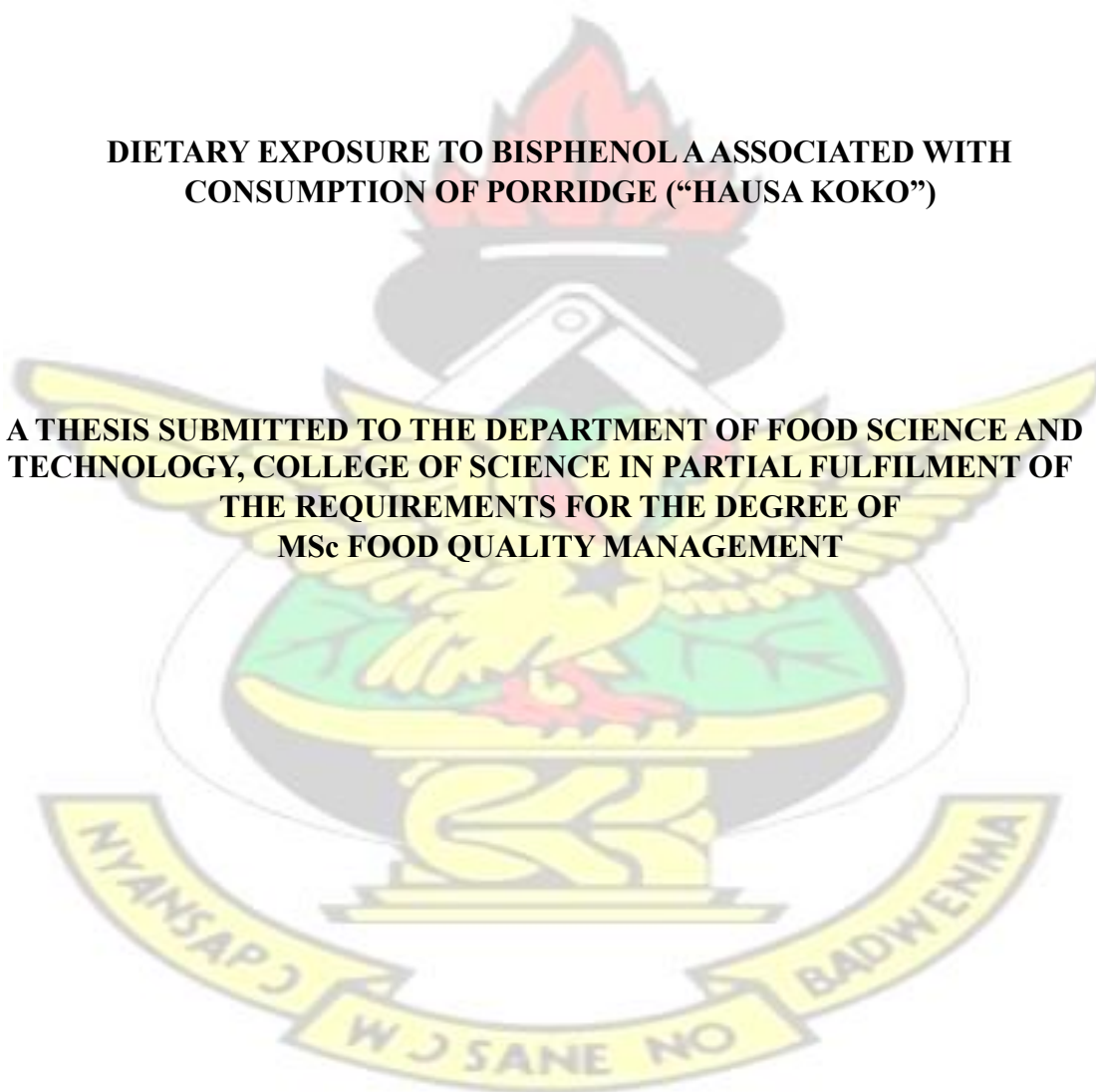


**KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY
KUMASI**

**COLLEGE OF SCIENCE
FACULTY OF BIOSCIENCE
DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY**

**DIETARY EXPOSURE TO BISPHENOL A ASSOCIATED WITH
CONSUMPTION OF PORRIDGE (“HAUSA KOKO”)**

**A THESIS SUBMITTED TO THE DEPARTMENT OF FOOD SCIENCE AND
TECHNOLOGY, COLLEGE OF SCIENCE IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MSc FOOD QUALITY MANAGEMENT**



SEPTEMBER, 2016

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DECLARATION

I hereby declare that this submission is my own work towards the MSc and that, to the best of my knowledge, it contains no material previously published by another person, nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

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ACKNOWLEDGEMENT

I am grateful to the Almighty God for His strength, grace, mercy, favour, love and protection throughout my education till now. I am greatly indebted to my supervisor, Mrs. G.M. Ankar-Brewoo firstly for her patience, tolerance and encouragement and secondly for her constructive suggestions and criticisms in the preparation of this report.

I'm equally indebted to Mr. I.W. Ofosu for his guidelines and suggestions.

My gratitude also goes to my ever-supportive wife, Abigail Attah and my children Kaylah Agyakoma Opoku and Jeremy Adutwum Opoku Jnr. To my mother Miss Grace Fenteng and my sister Sandra Opoku Konadu, God richly bless you guys and replenish everything you have lost in your quest to see your only son and only brother respectively climb high the ladder of education.

Finally, I will like to thank Barry Kojo Whyte and William Appau for their support in diverse ways to make this project work a success.



ABSTRACT

Bisphenol A (BPA), a member of diphenylmethane and bisphenols with two hydroxyl groups is used to produce certain types of plastics that are used in packaging food products. Containers made with these plastics may expose people to small amounts of BPA in food. 'Hausa koko' is a breakfast meal, packaged in transparent plastic film and popularly consumed in Ghana. The porridge is served at a temperature likely to enhance migration of bisphenol A from the transparent plastic film. This research sought to determine the levels of bisphenol A in the 'Hausa koko' and establish the risk of consumption. A cohort study using five vendors was used in this study.

Structured questionnaires were used to assess consumption characteristics of 'Hausa koko' by 320 respondents. A modified QuEChERS method was used for extraction and clean-up of BPA from 120 samples of "Hausa koko". A Cecil-Adept Binary Pump HPLC coupled with Shimadzu 10AxL fluorescence detector (Ex: 230 nm, Em: 316) with Phenonix C18, 5 μ m, 3.9 x 150 mm column was used at column temperature maintained at 40 °C to determine the level of BPA in 'Hausa koko'. Palisade @ risk software was used to determine the hazard quotient (HQ), of consumption of 'Hausa koko' to characterize risk. Results from the study indicated that bisphenol A leached into the food product at concentration of BPA ranging from < 0.005 ng/ml (below limit of detection) to 1.92 ng/ml. The rate of migration was affected by temperature, pH, storage time and sugar concentration. The HQ of BPA in "Hausa koko" with or without sugar was <1 indicating that consumers within the study population were at no significant health risk.

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CHAPTER ONE

INTRODUCTION

1.1 Background

Bisphenol A (BPA), has been used as a chemical in high volumes in the production and synthesis of plastics. As a carbon-based synthetic compound, it is a member of diphenylmethane and bisphenols with two hydroxyl groups. It is a colourless solid which aside dissolving in organic solvents is poorly soluble in water. They have however been used commercially since 1957 (Niu, 2015).

BPA is a chemical agent found in products that are used in our daily activities. It is employed in the plastic industry due to its cross-linking abilities with other chemicals. In view of this, BPA is a constituent of plastics including materials that are in contact with food such as plastic films used to package food like porridge, bottles used by babies, sippy cups and containers for food storage. It is also used to make protective coatings and linings for food and drink cans.

BPA exposure is linked to a variety of detrimental wellbeing such as endocrine disruption (vom Saal *et al.*, 1998), diabetes mellitus (Shankar and Teppala, 2011), obesity and insulin resistance (Wang *et al.*, 2011) and neurological effects (Palanza *et al.*, 2007). Consumption of BPA can also lead to an increased incidence of breast cancer (Briskin *et al.*, 2008) and brain tumors (Duan *et al.*, 2012). Studies conducted by Barr *et al.*, 2007 concluded that the effects of BPA are more pronounced in children, infants and fetuses since they lack mature organ systems and fully developed detoxifying enzymes to rid their system of such xenobiotics.

“Koko” is the product of a cereal processed into porridge in Ghana. It is made mostly from maize and millet. “Hausa Koko” is one of such porridges produced in Ghana. It is a product of the pearl millet also known as *Pennisetum glaucum*. As a ready to eat breakfast cereal, “Hausa Koko” is normally sold in the morning. This product is normally displayed in front of the residence of the seller or in an open market. In Africa, cereal based foods are used as complementary foods for children and infants. Their nature however, does not permit them to be used solely as complementary foods. This is as a result of their nutrient density and low levels of energy they provide (Lorri and Svanberg, 1994; Dewey and Brown, 2003; Lutter and Dewey, 2003).

Food safety involves the protection of food supply from microbial, chemical, and physical hazards that may occur during all stages of food production including growing, harvesting, processing, transporting, retailing, distributing, storing, preparing and consuming to prevent food borne diseases (WHO, 2007). That is to say, the components of food or the material used in packaging the food should not render the food harmful or injurious to the consumer.

In Ghana, transparent plastic films are also used in packaging ‘Hausa koko’ a milletbased porridge. Although information available in the current literature suggests that BPA, a constituent of plastic film, can leach into food, a data gap exist on the concentration of BPA in “Hausa koko” packaged in transparent plastic film. Again the risk associated with consuming ‘Hausa koko’ packaged in transparent plastic film is not known.

1.2 Justification

It has been established that Bisphenol A in foods and beverages make up about half (50%) of the human internal contact. The exposure of Bisphenol A into foods and liquids is made possible by two mechanisms. The first is as a result of the release of non-polymerised monomers. The other means is by the decay of polymer bonds. This results in the release of monomer bonds into the products. A study by EFSA (2015) revealed that infants and toddlers are more exposed to bisphenol A. The average concentration of Bisphenol A was found to be 375 ng/kg body weight per day and a highest estimated dietary exposure of 857 ng/kg body weight per day for toddlers below six months. The research again found the modelled dietary exposure for teenagers, adults and the elderly to have been in the range of 116 ng/kg to 159 ng/kg. Their exposure however, was in the ranges of 341 ng/kg and 388 ng/kg.

In view of the wide spread human exposure and the health effects associated with BPA, there is a growing consumer concern about the safety of transparent plastic film used in packaging “Hausa koko” due to possible migration of BPA, particularly when “Hausa koko” is packaged at high temperature for the consumer when sold. Hence, there is an urgent need to sample and analyze for the presence of BPA in “Hausa koko” packaged in transparent plastic film.

1.3 Objectives

The general objective of this study was to understand the toxicological impact of BPA in the trend of plastic packaging of “Hausa koko”. The specific objective was to determine the concentration of BPA in “Hausa koko” sold in transparent plastic films

and subsequently determine the risk associated with consumption of the “Hausa koko” product.

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CHAPTER TWO

LITERATURE REVIEW

2.1 Bisphenol A

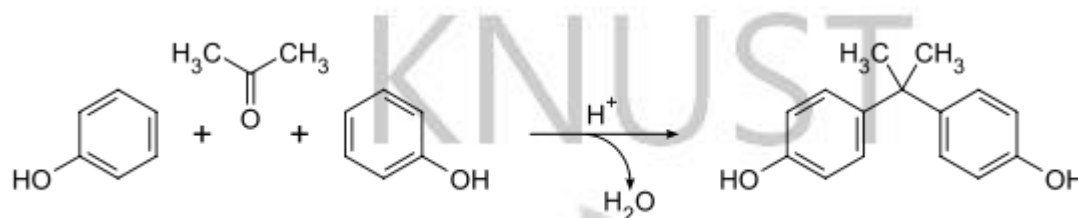
Bisphenol A has a chemical formula of $\text{HO-C}_6\text{H}_4\text{-C}(\text{CH}_3)_2\text{-C}_6\text{H}_4\text{-OH}$. This chemical although a monomer was initially synthesized as an artificial estrogen. It has become prominent because of its ability to form cross-links with other chemicals (Chemical fact sheet, 2012). The properties of bisphenol A enable it to be used as a production raw material for products such as plastic polymers and epoxy resins. These products are used for the manufacture of food and beverage containers and sealants in dental products. These are all made possible because of the properties of the chemical (Alonso – Magdalena *et al.*, 2006). Production of bisphenol A stands tall among other chemicals, because of its wide usage. More than 6.4 billion pounds of BPA were produced in the year 2003 worldwide. Its annual growth rate has been estimated between 6 to 10 % (vom Saal *et al.*, 2005).

Humans continue to be exposed to this chemical because of its use. It is used to formulate polycarbonate plastics. It is again used to line cans in most countries in the developed world (Calafat *et al.*, 2005). It is used in such products for basically two reasons. In the first place it has been established that when food that is acidic or basic in nature or food that has been heated comes into contact with plastic material can result in leaching of bisphenol A from the plastic material into the food matrix, (vom Saal *et al.*, 2005). The second reason is its ability to disrupt the functions of the endocrine (vom Saal *et al.*, 1998). By so doing it is able to stop or act as an estrogen. This has been reported in studies involving the use of laboratory animals. This problem was encountered even when the doses of bisphenol A presented to the animals were low in dose (vom Saal *et al.*, 2005). Laboratory animals are used as subjects in place of humans in laboratory tests. The results obtained add on to knowledge that the presence of BPA in the human body is a requisite for disaster.

They can change the balance in the human body.

2.2 Physical and chemical characterization

Bisphenol A is an organic chemical. It is produced by a reaction between phenol and acetone. An acid catalyst is used to catalyze this reaction. It has the chemical formula $C_{15}H_{16}O_2$. The molecular mass of bisphenol A is 228.29 g/mol (Niu, 2015).



Plat 2.1: Production of Bisphenol A

It has a chemical abstracts service number of 80-05-7 and EC No 201-245-8 (European Chemical Substances Information System (EINECS) number). Its IUPAC name is 4,4'-Dihydroxy-2,2-diphenylpropane. It is also known as 2,2-bis(4Hydroxyphenyl)propane or 4-[2-(4-Hydroxyphenyl)propan-2-yl]phenol. Bisphenol A is also known in other sectors as bisphenol A, Bis (4-hydroxyphenyl) dimethyl methane, 4,4'-Dihydroxydiphenyl propane and Diphenylolpropane.

Lewis (2001) and O'Neil (2006) have described bisphenol A as a white solid which is available in crystalline form or as flakes. In the presence of dilute acetic acid, they crystallize as prisms while they appear as needles in the presence of water (Lide and Milne, 1994). During normal room temperature they are known to produce mild phenolic odour (O'Neil, 2006). Bisphenol A melts within a temperature range of 150–158 °C. It also boils within a temperature range of 360–398 °C (at 101.33 kPa, (IUCLID, 2000; Cousins *et al.*, 2002). The density of BPA is 1.195 kg/dm³ at 25 °C (IUCLID, 2000; Lewis, 2001). It has a vapour pressure of 5.3 x 10⁻⁶ Pa at 25 °C (Cousins *et al.*, 2002).

BPA is a moderately hydrophobic compound and has an octanol–water partition coefficient ($\log P_{ow}$) of 3.32 (Hansch *et al.*, 1995). The two hydroxyl groups make it a little polar. BPA is soluble in a number of compounds. These include acetic acid (Lide and Milne, 1994), aqueous alkaline solution, alcohol, acetone (O’Neil, 2006), benzene and diethyl ether (Lide, 2004). In water its solubility is 120–300 mg/L at 25 °C (Cousins *et al.*, 2002). It also has a pKa value of between 9.59 and 11.30 (Cousins *et al.*, 2002). The pKa value makes BPA remain in its non – ionized form in liquid media. This is possible even in pH values below 7. The BPA molecule has a fairly strong fluorophore and it can be detected by its fluorescence. Its chromophore is relatively weak, and the sensitivity of ultraviolet (UV) detection is much lower than that of fluorescence detection (Stephen, 2000). BPA does not persist in the environment, although it is fairly stable in its solid form. Aerobic biodegradation is the dominant loss process for BPA in river water and soil, with a degradation half-life of approximately 4.5 days (Cousins *et al.*, 2002). Its loss process in the atmosphere is due to the rapid reaction with hydroxyl radicals, and the photo-oxidation half-life for BPA in air is about four hours (Cousins *et al.*, 2002)

Table 2.1: Physical characterization of BPA

Parameter	Specification
Log K_{ow}	3.40
Boiling point	220 °C (4 mmHg)
Melting point	150–155 °C
Water solubility	120–300 mg/l

Source (Staples *et al.*, 1998)

Table 2.2: Characterization of BPA

Parameter	Specification
Molecular formula	$(CH_3)_2C(C_6H_4OH)_2$
Molecular weight	228.29
LD50, rat, oral	3300–4240 mg/kg

LD50, mouse, oral	2500–5200 mg/kg
LD50, fish (<i>pimephales promelas</i>)	96 h 4.6 mg/l
LOAEL	50 mg/kg/day
Reference Dose	50 µg/kg/day

Source (Staples *et al.*, 1998; US EPA, 2010)

2.3 Exposure to Bisphenol A

As a result of increase in the use of plastics such as polycarbonate in food packaging, exposure to bisphenol A by humans has also increased. Although the environment including aquatic, air and soil are known to be sources of human exposure to BPA, the major route of human exposure is diet (Kang *et al.*, 2006). Humans are exposed to Bisphenol A mainly through foods and beverages. When compared to teenagers, adults and the elderly, infants and toddlers of ages above 6 months are more exposed to BPA for a given population (EFSA, 2015). The estimated average BPA exposure through diet in infants and toddlers is 375 ng/kg body weight/day with 875 ng/kg body weight/day estimated as the highest dietary bisphenol A exposure (EFSA, 2015). On the contrary, average dietary exposure to BPA in teenagers, adults and the elderly range from 116 to 159 ng/kg b.w./day with a range from 341 to 388 ng/kg b.w./day being the range for high exposure (EFSA, 2015). The abundance of the presence of bisphenol A in nature in most developed countries makes humans exposure very possible. It is therefore no news that BPA is found in detectable concentrations in the human body. From a study conducted in the United States of America, bisphenol A concentrations were found in about 95% of the test urine samples (Calafat *et al.*, 2005). These results were confirmation of similar studies conducted in countries such as Japan and South East Asia. The presence of BPA in the samples confirmed that BPA leaches into food and water.

Volkel *et al.* (2002) worked on the metabolism of BPA in the human body. Results from their findings concluded that BPA is excreted in the urine within 24 h of administration. The excretion experiment has led scientists and researchers to believe that humans are more exposed to BPA than previously anticipated (Volkel *et al.*, 2002; 2005). They claim that huge amounts need to be ingested before they can be detected in urine of man.

2.4 Metabolism of Bisphenol A

Analysis of total unconjugated and conjugated plasma-concentration time profile following oral and intravenous administration of BPA in rats and monkeys indicates that bisphenol A is rapidly absorbed from the gastrointestinal tract (Farabollini, 1999). Studies conducted in humans in whom BPA was administered orally at a relatively low dose indicated a complete absorption of BPA from the gastrointestinal tract (EURAR 2003; 2008). Results from a study conducted by (Völkel *et al.*, 2002) concluded that metabolism of bisphenol A occurs primarily in the liver and partially in the gut. The study further indicated that following oral ingestion of bisphenol A, majority of the chemical binds to glucuronic acid in a metabolic process called glucuronidation to produce bisphenol A-glucuronide. This process is mediated by enzymes (UDPglucuronyltransferases) in the liver. Bisphenol A-glucuronide compared to the unconjugated parent bisphenol A is more soluble in water and hence it's easily eliminated in the urine with terminal half-lives of less than 6 h (Völkel *et al.*, 2002). Other studies conducted in humans revealed an additional sulfotransferase mediated sulphate conjugation to form BPA-sulphate; a minor metabolite following oral absorption of bisphenol A with similar observations made from studies conducted in rodents (EFSA, 2006). Where as in humans and monkeys the predominant way of eliminating conjugated bio-transformed bisphenol A is through the urine, in rodents

both the parental and conjugated bisphenol A is predominantly eliminated through feces (EFSA, 2015).

2.5 Migration of Bisphenol A

Migration involves the movement of substances from one location to the other. The manufacture of plastics using bisphenol A as a monomer involves the creation of ester bonds. Under conditions such as heat and in the presence of acidic or basic compounds, the ester bonds are susceptible to hydrolysis. The repeated use of plastic materials also presents this same situation (Vom Saal and Hughes, 2005). By this, the plastic breaks down. It then leaves the monomers of BPA to build up in the containing vessel (vom Saal and Hughes, 2005). Because of the vulnerability of babies, studies have been conducted on the concentration of BPA in baby bottles, food storage cans, and water bottles (both for use by people and laboratory animals) (Brede *et al.*, 2003; Consumers Union, 1999; Goodson *et al.*, 2004; Howdeshell *et al.*, 2003). The main objective of the experiments was to establish the migration level of BPA in the human environment. This was carried out by altering the environmental conditions. The responses to the conditions were measured.

Increases in the migration of BPA into food and water were found in the following conditions; increases in heat, acid, base, age and usage of plastic products (Brede *et al.*, 2003; Consumers Union, 1999; Goodson *et al.*, 2004; Howdeshell *et al.*, 2003; Kang *et al.*, 2003). The presence of BPA in products makes these results very important to science and humans. BPA is found in products including but not limited to linings of beverage cans, sealants used by dentists, polycarbonate plastics, water bottles and baby bottles (Calafat *et al.*, 2005). Some producers have attempted to label plastics which included BPA in their manufacture. Such bottles are labeled as number

7. This is normally displayed at the bottom of the bottles (Howdeshell *et al.*, 2003).

2.5.1 Leaching of Bisphenol A from polycarbonate materials

The diverse nature of BPA in the environment makes them to be exposed to human beings. Since it is part of the objects humans use in their daily activities, they become easily exposed to them. Each person consumes one food or the other. This is one of the major routes of BPA exposure in man. They are exposed as a result of consumption of contaminated food that has come into contact with BPA. Production of plastics has increased over the years in the world. They have therefore been used in packaging various products. These include consumer products. Their production is known to contaminate the environment. Le *et al.* (2008) studied the migration of BPA from polycarbonate plastics into food and water. Their results concluded that BPA migrates from the plastics into food and water. They explained that, the migration might have been as a result of the breakdown of polymers. Some studies which were meant to raise general concern on BPA included studies on baby bottles. They studied the possibility of BPA leaching into baby bottles (Vandenberg *et al.*, 2007). Today, chemical analysis is used by researchers to study the migration of BPA from industrial plastics. Water is consumed by every person in the world. In both the developed and developing countries, water is consumed in plastic containers. The migration of BPA from these bottles is also under study.

The release of bisphenol A from polycarbonate drinking bottles has been studied by Le *et al.* (2008). They further compared the mechanism of chemical migration from brand new and used bottles. Conditions for consumer usage of the bottles were modeled. These were done in the right temperatures and conditions. Migration of bisphenol A at room temperature and boiling temperature were examined. The studies concluded that

bisphenol A leaches from all the polycarbonates used. The concentration of leach of bisphenol A increased with increase in time at both room and boiling temperatures. The concentration of the leach of bisphenol A in used and new bottles were about equal. Results from Le *et al.* (2008) recorded a maximum migration of 7.67 µg/ml.

2.5.2 Polycarbonates (PC) used in baby bottles

BPA can be moved from PC into foods by diffusion of residual BPA present in the polymer after the manufacturing process. Bisphenol A can also leach into baby bottles through breakdown of ester bonds. This reaction is catalyzed by the hydroxide ion when the polymer is in contact with the aqueous food (Mountfort *et al.*, 1997; Hoekstra and Simoneau, 2013). In other studies, they attribute release of BPA to the control of diffusion. They confirm that hydrolysis of the polymer chains is the main reason behind the release of BPA (Biedermann-Brem *et al.*, 2008; Biedermann-Brem and Grob, 2009; Mercea, 2009). In other studies, it was found that the migration of BPA from PC plastics had no linkage with the residual concentration left behind during the process (Mercea, 2009). This goes to confirm that a transfer takes place as opposed to diffusion.

A number of studies have been undertaken to identify the factors which influence BPA migration. Temperature, time and re – use of bottles are some of the factors studied (De Coensel *et al.*, 2009; Kubwabo *et al.*, 2009; Mercea, 2009). The pH condition of water is known to be of relevance when studying the release of BPA in boiling conditions. During heating, carbon dioxide from the water is evaporated. This increases the pH to a value of up to about 9. This pH has been studied and known to accelerate the release of bisphenol A. Because of this mechanism, it is important to ignore the addition of distilled water to test samples (Biedermann-Brem and Grob, 2009).

The effect of PC ageing was investigated by Le *et al.*, 2008, Kubwabo *et al.*, 2009 and Mercea, 2009. In an attempt to test the level of migration of biphenol A under heat, two methods were adopted; migration in water bath and migration under microwave. Results from this study concluded that although temperature influences rate of migration, under these two conditions no significant differences were observed in the migration rates (Ehlert *et al.*, 2008).

A review has been done on the release of bisphenol A from PC bottles (Hoekstra and Simoneau, 2013). Baby bottles have been a major area of concern in the work on BPA migration. Simoneau *et al.* (2011) analyzed concentrations of BPA in baby bottles in some selected European markets. The results from the study concluded BPA < LOD (0.1 µg/kg) in 32 out of 40 PC baby bottles when tested with 50 % ethanol for two hours at 70 °C after boiling for 5 min. Highest migration value was 1.83 µg/kg. Second and third tests were carried out. Results from the second and third tests confirmed that most of the bottles did not release detectable levels of BPA carried out with the simulant.

Santillana *et al.* (2011) studied the migration of BPA in 72 PC baby bottles from 12 different brands collected in the Spanish market. Conditions set for the migration included a solution of 50 % ethanol and 3 % acetic acid, for 2 h at 70 °C and followed by 24 h at 40 °C. The results from the study concluded that 18 µg/kg of BPA were leached into 3 % acetic acid (Santillana *et al.*, 2011). In other studies the migration of BPA in water increased. This increase has been attributed to the effect of residual alkaline detergents. These are left over chemicals found on dishwashers after washing the plastics (Biedermann-Brem *et al.*, 2008; Biedermann-Brem and Grob, 2009; Maia *et al.*, 2009). The levels of BPA on the dishwashers are indications of the need to ensure high standards of cleaning. Baby bottles therefore need to be washed well to prevent

the incidence of residual alkaline materials. Another study by Kubwabo *et al.* (2009) was on the migration of BPA from PC plastic. PC reused drinking bottles and liners for baby bottles a total of 24 baby bottles, 10 liners for baby bottles (HDPE and LDPE), 5 new and 5 old (used beyond 5 months) were used for the study. This work involved varying conditions needed for migration. The test samples were kept under 40° C for 10 days. The concentration of BPA in the PC baby bottles was found to be 1.88 µg/kg in water and 2.39 µg/kg in 50 % ethanol. The results also found significant differences between old and new bottles. Values obtained for the new and old bottles were 0.01 and 0.2 µg/kg, respectively (Kubwabo *et al.*, 2009). Le *et al.* (2008) reported different results on the use of new and old bottles. Their results recorded no significant difference in the bottles used. Their results concluded that migration of bisphenol A is independent of the nature of the bottle used. Values recorded for new and old bottles in their work at room temperature are; new (1.0 µg/kg) and used (one to nine years) PC bottles (0.7 µg/kg).

Aqueous food stimulants are also extensively used. The concentrations of BPA into aqueous food stimulants were studied for baby bottles. These studies were done to establish the effect of cleaning in a dish washer, cleaning with brush and sterilization in boiling water on the migration of bisphenol A. After the study, washing with brush had no impact on the migration of BPA. The temperature of the water used was however seen as a critical factor in the migration process. The role of temperature is a confirmation of results by other authors on migration of BPA under various temperatures. The concentrations of BPA in the test samples ranged from 2.4–14.3 µg/kg when filled with boiled water and left at ambient temperature for 45 min. After repeated use, the concentrations in BPA decreased in the sterilized water (Maragou *et al.*, 2008).

A survey was conducted on chemicals that migrate into foods. Food materials used for the study included baby bottles made from PP, PES or silicon (Simoneau *et al.*, 2012). Results showed that samples such as baby bottles made of PP, PES or silicone had bisphenol A concentrations. Bisphenol A was however detected in some samples of two models of polyamide baby bottles produced in the Netherlands and Switzerland. The concentrations of BPA in the samples were within the range of 1 to 329 $\mu\text{g}/\text{kg}$, with an average value of all data (including non-detects) of 25 $\mu\text{g}/\text{kg}$ in the third migration test. Migration values of 1.08 $\mu\text{g}/\text{kg}$ were detected for the first migration tests in a bottle. After a repeat of the test, an accidental illegal presence of BPA was reported. The calculations used in reporting exposure rates were based on consumption frequencies. Test samples were assumed to ingest the product 6 times in a day for 90 days. When this was done, the rate of migration decreased by about 80 % after the third migration. A linear decrease was assumed, which meant falling below the LOD (0.1 $\mu\text{g}/\text{kg}$) between the third and the sixth use (i.e. day 1). The simulation was based on the experimental value from migration into 50 % ethanol as simulant. It led to an average estimate of 0.45 $\mu\text{g}/\text{kg}$ food and the 95th percentile was 1.24 $\mu\text{g}/\text{kg}$.

2.5.3 Coatings, caps, closures and other

Bisphenol A is also known to migrate in coatings, caps, closures and other materials which perform such operations. Bradley *et al.* (2007) studied the migration of BPA from coatings used in cooking ware. In their work olive oil was heated at a temperature of 175 °C for 30 min. The concentrations of BPA migrated were below 6 $\mu\text{g}/\text{kg}$ after the third reuse with olive oil at 175 °C for 30 min. They confirmed that the concentrations were bound to reduce on sequential contact periods.

Fasano *et al.* (2012) studied the migration of BPA in 11 packaging materials used for food products. The packaging materials included tuna cans, caps of marmalade jars, several plastic packages, bread bag, LPDE film, baby bottles, aseptic plastic laminated paperboard carton and two plastic wine tops. Results from the study concluded that the highest level of migration was in plastic wine tops although wine tops are not in contact with the wine but in the headspace of the bottle. In other containers, the compounds detected may reflect the composition of the material, although in many instances it is difficult to determine the origin of phthalates, alkylphenols and DEHA since knowledge on the type and amounts of plasticizers and additives added during the manufacturing of plastics and containers is unspecified. This study provides information on the release of compounds which have endocrine disrupting properties.

2.5.4 Effects of temperature on migration of BPA

Temperature is found to be one of the factors essential for the migration of BPA. Le *et al.* (2008) studied the effects of temperature on new and used bottles. The researchers concluded that the concentrations of BPA in heated samples are twice those in room temperature. A linear relationship is thus found between BPA and temperature. The higher the temperature the higher the concentration of BPA migrated. The researchers justified their study by claiming that during cold weather hot water is stored in bottles. The work again found an elevated rate of BPA migration to have been achieved after hot bottles were cooled and incubated with room temperature water. This phenomenon is meant to explain that the leach of BPA is not only limited to heating but long term effects also take place (Le *et al.*, 2008).

The properties of polycarbonate plastic make it the ideal material to be used in the production of plastics. It is transparent, has good wear resistance and stable under sterilization. After washing bottles in dishwashers, boiling them and brushing,

researchers found increases in the migration of BPA (Brede *et al.*, 2003). The researchers tested and found BPA concentrations in 12 new bottles. After subjecting them to various treatments such as dishwashing, boiling and washing with brush significant increases were observed in the concentrations of BPA. Concentration of BPA decreased between 51 washings to 169 (Brede *et al.*, 2003). These results are a confirmation of work by Le *et al.* (2008), who found no significant differences between used and new bottles.

Another team of researchers studied migration of BPA in baby bottles. They however used human participants (Maragou *et al.*, 2008). They examined migration of BPA using liquid chromatography. In all 21 baby bottles were used in the study. They were studied in water or an aqueous food substance. The bottles were cleaned with brush, dish washed and sterilized. At the end of the study, temperature was found to have significantly affected the results.

Howdeshell *et al.* (2003) conducted studies on migration of bisphenol A using animal cages. They studied the migration under different temperatures, using new and used cages. The concentrations were higher in elevated temperatures and lower at room temperature. Their work again measured the migration at room temperature and at neutral pH. The researchers incubated purified water at normal room temperature in new cages, used cages and glass containers (control.) The results concluded that significant amounts of BPA were released from used cages, a little were released in the new cages and none released in the glass bottles. The results are clear indications that animals stored in such cages are at risk of being exposed to BPA.

The study by Le *et al.* (2008) acknowledges the difference in findings from their study (Howdeshell *et al.*, 2003) about the increased migration of BPA after use. The main

cause for such findings was because hot water with temperatures of 82 °C was used in cleaning the cages. They thus practiced the laws of Association for Assessment and Accreditation of Laboratory Animal Care standards. In our homes however bottles are washed at temperatures within 50 °C (Le *et al.*, 2008).

2.5.5 Effects of glucose, sodium chloride and date of expiration on migration of BPA

Sungur *et al.* (2014) conducted a study on the effects of glucose, sodium chloride and expiration date on migration of Bisphenol A into foods packaged in containers whose inner surface had been lined with plastic film, foods in glass jar and metal cans displayed for sale on the Turkish market. The study concluded that changes in the amount of bisphenol A in all foods are based on date of expiration, the amount of glucose and sodium chloride. They further concluded that the amount of bisphenol A increases with an increase in the amount of glucose, NaCl and expiration date.

2.6 Effects of Bisphenol A

2.6.1 Endocrine-disrupting chemical

Bisphenol A plays an active role in the disruption of the endocrine system of both man and animal. They have been referred to as endocrine disrupting or hormonally active chemicals. Their mode of operation requires them to attach themselves to the receptor sites for hormone production (vom Saal *et al.*, 1998). They thus present themselves as the hormones and work to destroy its activity. They are specifically known to disrupt the endocrine hormone. They have the ability of attaching themselves to the estrogen receptors in the body. Their action has however been found not to be detrimental to adults. It is however a fatal case when they are found in developing fetus (vom Saal *et al.*, 1998). Adults are presented with hormonal imbalances. This affects some vital

functions in their body. In contrast, an animal that is undergoing a key developmental period, such as while in the womb, is much more susceptible to changes in the hormonal balance (vom Saal *et al.*, 1998).

vom Saal *et al.* (1998) studied the effects of bisphenol A in the reproductive organs of laboratory mice. The study involved the use of pregnant female laboratory mice. They were presented with two separate doses of bisphenol A for a period of 7 days. The doses included 2 ppb and 20 ppb. When the pregnant females delivered, their little ones were left to mature. The male mice were separated and randomly selected for the study. The doses used had significant effects on the reproductive ability of the male mice used. The size of the mice preputial glands were increased by about 35 % after being fed on the 2 ppb level. The preputial glands are responsible for the socio-sexual behaviors exhibited by males, specifically urine marking and aggressiveness towards other males (Vom Saal *et al.*, 1998). The epididymis was however reduced after being on the 2 ppb concentration. This organ is used to store sperms. The size of the seminal vesicles, an organ which contributes fluid for ejaculation was also reduced. This organ is known to be an important tool in fertility in male mice. This happens because the two organs are found on separate sides of the embryonic tissues in mice. The epididymis and seminal vesicles both develop from Wolffian ducts; while the preputial glands develop from tissues in the embryonic urogenital sinus and perineum (other organs which develop from these tissues include the prostate, penis, and scrotum (Vom Saal *et al.*, 1998). Both the Wolffian ducts and the urogenital sinus and perineum contain estrogen receptors during prenatal development in mice, and can therefore be affected by any abnormal amounts of estrogen (Vom Saal *et al.*, 1998).

The efficiency in the production of sperms decreased by 20 % for the mice which were on the 20 ppb BPA concentration. In a similar work by the same authors, prostate glands

of mice exposed to 2 ppb and 20 ppb were increased in the margin of 30 % when compared to the control sample mice (Vom Saal *et al.*, 1998). These results confirm that changes in the concentration equally have an effect on the reproductive system. The results could however not confirm if the situation was worse when one took higher doses.

2.6.2 Low dose effect

There is a growing concern regarding the human health effects of bisphenol A even at low doses. Low dose effects of a chemical refer to responses that occur at doses well below the traditional lowest dose used in toxicological studies for risk assessment purposes. US EPA has established 50 mg/kg/day as BPA lowest dose for risk assessment purpose. This lowest dose also known as lowest observed adverse effect level (LOAEL) was used to calculate the current EPA reference dose or acceptable daily intake (ADI) dose by dividing the LOAEL by safety factors of 1000. Hence the US EPA (2010) established reference dose for BPA is 50 µg/kg/day. Report from studies conducted by Vom Saal and Hughes, (2005) indicates 94 out of 115 published *in vivo* studies relating to low dose effects of BPA reported significant effects.

A comprehensive review of literature reveals that brain structure, brain chemistry, blood hormonal levels, behavior, enzyme activity, growth rate and sexual maturation are significantly affected by bisphenol A exposure at low doses (Vom Saal and Hughes, 2005). Report from various published toxicological studies conducted on BPA indicated significant health effects occurred below the reference dose of 50 µg/kg/day. An increased postnatal growth in females at maternal doses of bisphenol A between 2.4 and 500 µg/kg/day was reported from a study on the effects of maternal xenoestrogen exposure on development of the reproductive tract and mammary gland in female mouse offspring (Nikaido *et al.*, 2004). Early onset of sexual maturation was also

observed in females at maternal doses between 2.4 and 500 $\mu\text{g}/\text{kg}/\text{day}$ (Honma *et al.*, 2002). A study conducted by Akingbemi *et al.* (2004) reported level of plasma luteinizing hormone in rat Leydig cells were altered at a maternal dose of 2 $\mu\text{g}/\text{kg}/\text{day}$. Significant disruption of chromosomes during meiosis in developing oocytes during puberty were reported in a study conducted in female mouse (Hunt *et al.*, 2003). The study indicated that the observation was due to leaching of bisphenol A from polycarbonate drinking bottles at doses between 15 and 70 $\mu\text{g}/\text{kg}/\text{day}$. Other toxicological studies also reported altered immune function in mice at doses between 2.5 and 30 $\mu\text{g}/\text{kg}/\text{day}$ (Sawai *et al.*, 2003; Yoshino *et al.*, 2004).

Sugiura-Ogasawara *et al.* (2005) concluded that women who are exposed to higher levels of BPA than a control group were more likely to have recurring miscarriages. Patients were found to have 2.59 ng/mL of BPA in their blood, where the control groups were found to have 0.77 ± 0.38 ng/mL of BPA in their blood. It was also found that women with autoimmune diseases tend to have recurring miscarriage upon exposure to BPA. A more recent study found that prenatal exposure to environmentally relevant doses of BPA may result in morphological and functional alterations of male and female genital tract and mammary glands that could predispose those tissues to earlier onset of disease, reduced fertility and mammary and prostate cancers (Kuruto-Niwa *et al.*, 2007).

A study on adult male sexual dysfunction on subjects who work in a factory that manufacture Bisphenol A concluded that the workers were four times (4x) more likely to report of erectile dysfunction. The workers also had reduced sexual desire. The workers were again reported to have reduced sexual function within one year of employment (Li *et al.*, 2010). The adverse effects of bisphenol A at low doses in animal experiments raise concern with regard to human health upon exposure to BPA.

2.7 Production and packaging of “Hausa koko”

Fermentation has been used as a food processing and preservation method in Africa over the years. Products from fermentation include porridges, alcoholic and non alcoholic beverages, bread, fermented fish and dairy products (Campbell-Platt, 1994; Steinkraus, 1996). Specific fermented foods include kenkey, ‘momoni’ and “koko” in Ghana and Injera in Ethiopia. ‘Koko’ is the traditional name given to cereal based porridges produced in Ghana. They are mainly produced from maize or millet. “Hausa Koko” is produced from pearl millet also known as *Pennisetum glaucum*. It is made as a ready to eat food. Sellers of this product usually do so in front of their houses or in market places. The nutritional benefit attained from the consumption of millet has made it to be used as a complementary food (Owusu-Kwarteng, 2010). Biological processes of the ingredients used, the nutritional, physical and safety aspects associated with the production of such a food is of paramount importance to researchers (Mensah *et al.*, 1991; Mensah *et al.*, 2002).

The preparation of “Hausa Koko” (Figure 2.1) begins by measuring 5 kg of cleaned whole millet grains. This is soaked in about 8 L of water at room temperature (28-30 °C) for 12 h. After 12 h, the steep water is decanted. The wet millet grains are then wet milled in a plate attrition mill. A quantity of water (about 6 L) is added to the milled millet. It is kneaded to form dough. The dough is kneaded and sieved to remove the chaff. The filtrate obtained is left to settle. This process is also known as the fermentation stage. This takes place for about 12 h. The fermentation process is ended by separating the supernatant from the decanted paste. The supernatant is heated to boiling point and the decanted paste added while stirring, and cooked for about 3 min to form porridge. When cooked, it is stored in aluminum containers (Owusu – Kwarteng, 2010).

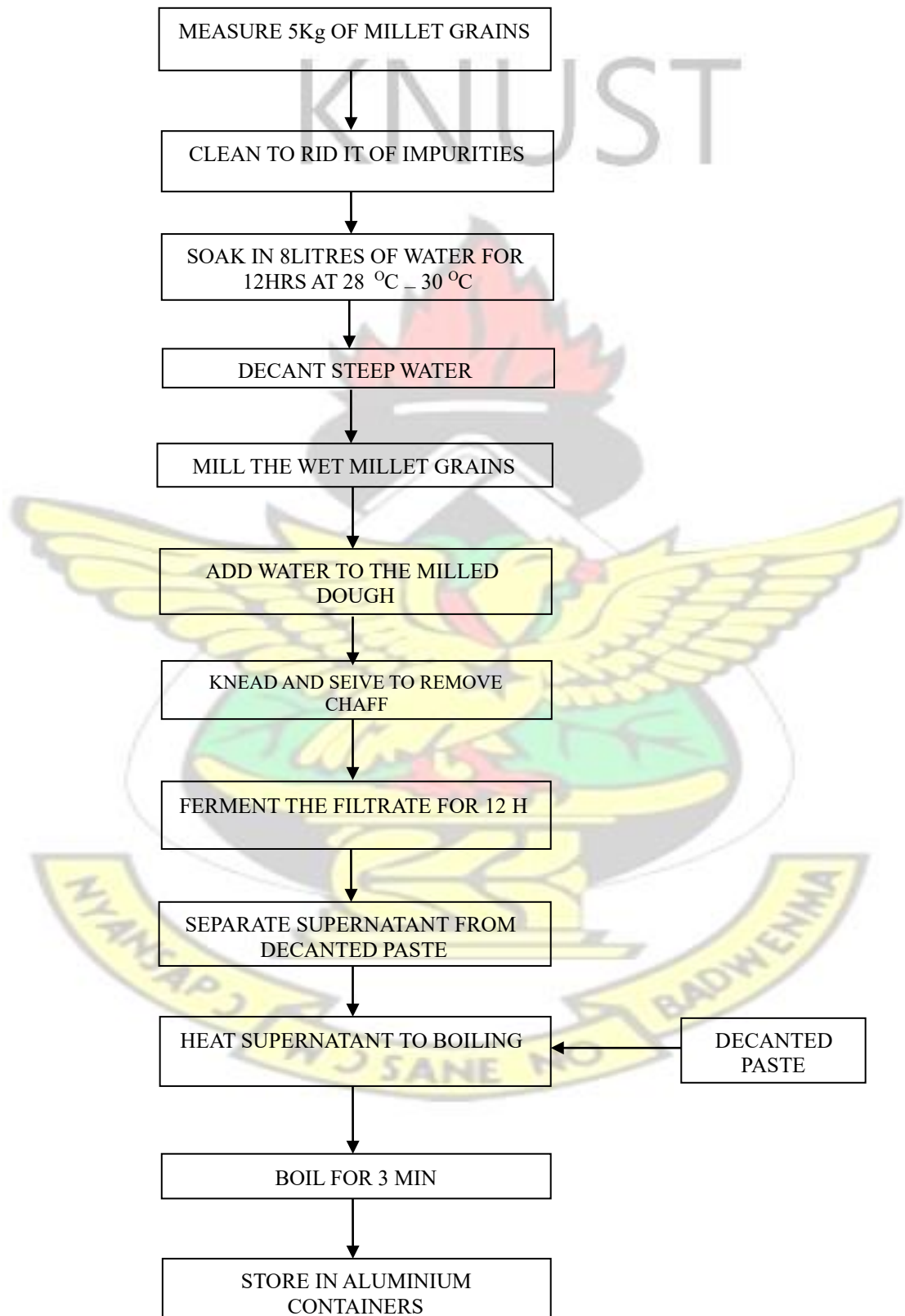


Figure 2.1: Process flow diagram for ‘Hausa koko’

2.7.1 Packaging of “Hausa koko”

Transparent plastic film formed into bags is becoming increasingly important in the packaging of a variety of foods. The adoption of these bags in packaging has significantly improved the display of ready-to-eat foods from aesthetic and hygienic points of view. Unfortunately, many food vendors are not familiar with the suitability or otherwise of the various types of plastic films for different products (Kellyn, 2010). This can lead to deterioration in the quality of the foods.

Transparent plastic films are manufactured locally and are available in different sizes, ranging from narrow strips of 3 x 5 cm to larger bags measuring 25 x 40 cm. These film wraps are desirable for packaging food because they are much less permeable to water vapour and gases than paper and leaves. They are used to package both solid and liquid foods. Transparent plastic films are useful for dry products such as gari, sugar and cocoa powder, as the items remain dry for a long time if properly sealed. Since heat-sealing devices are not readily available to many vendors, the open ends of the bags are usually tied into firm knots after the food is inserted (Owusu-Kwarteng, 2010).

CHAPTER THREE

MATERIAL AND METHODS

3.1 Materials

The porridge samples packaged in transparent plastic films and stainless steel containers (with and without sugar) were obtained from five (5) porridge sellers at Ayigya, Kumasi and stored in vacuum plastic container (to keep the temperature controlled) prior to analysis. Methanol, Toluene, Acetonitrile, Magnesium sulphate, Sodium chloride and Potassium phosphate used for preparation, extraction, purification and analyses were purchased from Sigma Aldrich Chemicals (UK).

Authentic standard of bisphenol A was obtained from Fluka (Sigma Aldrich, Germany). Oven, homogenizer, shaker, vortex, centrifuge and HPLC (A Cecil-Adept Binary Pump HPLC, UK coupled with Shimadzu 10AxL fluorescence, Japan) were purchased from Gen Lab, UK; IKA[®], Germany; Gallenkamp Orbital Shaker, UK; VortexGene, Holland; Eppendoff, USA, respectively.

3.2 Methods

3.2.1 Study area

Ayigya is a community in the Ashanti region of Ghana. It is about 5 min drive from the Kwame Nkrumah University of Science and Technology. Ayigya can be located on Latitude 6.687119 degree and Longitude -1.621969 degree. This locality is made up of people of various backgrounds. They include lecturers, students, christians, moslems, males, females, children, youth, the aged, bankers and the local indigenes. This area was selected for the research because of its nearness to the laboratory where analysis for the research was undertaken. The diverse category of persons located in this area also made the site an ideal site for the work.

3.2.2 Study population and selection

A total of 120 samples were used during the study period. The study was conducted between October and November, 2015.

A cohort study involving five (5) vendors was used for the study. The study was conducted for a period of two (2) weeks. Each vendor was visited once per week. A total of twelve (12) samples were taken from each vendor per visit per week. The 12 samples included; five (5) samples in transparent plastic film with sugar, five (5) samples in transparent plastic film without sugar, one (1) sample with sugar in stainless steel container (control), and one (1) sample without sugar in stainless steel container (control). A total of three hundred and twenty participants were recruited for the study to complete the questionnaires.

3.2.3 Study sampling and participation

A list of porridge sellers were identified from the study area. Five (5) of them were selected and used for the study. The selection criterion for the vendors was based on the number of years a vendor has spent in selling the product. The minimum number of years was chosen to be two (2). Rationale for the study was explained to the sellers. With the assistance of the vendors, participants were selected to undertake the study after giving their consent.

3.2.4 Dietary and lifestyle questionnaires

Participants were made to complete a 24 h self-administered food frequency questionnaire. First, the content of the questionnaire was explained to the participants. The consumers were made to give information on 'Hausa koko' packaged in transparent plastic film which they have consumed within 24 h from the time the questionnaire was administered to them. Various amounts of "Hausa koko" (GHC 0.5, GHC 0.6 and GHC 0.7, GHC 0.8, GHC 0.9 and GHC 1) were purchased from the vendors. The volume (mL) of each was measured (470, 520, 530, 580, 620, 680) and used as a standard volume for the study. The standard volumes were used as a basis for the quantities of "Hausa koko" ingested by the participants per day. That is the amount of 'Hausa koko'

packaged in transparent plastic film consumed per day in Ghana Cedis (GHC) was matched with the corresponding volume of 'Hausa koko' already determined. The participants were required to indicate the number of times they consume 'Hausa koko' in transparent plastic film per day. The questionnaire (see appendix) also required participants' weight to be taken. The questionnaire was pretested before the study.

3.3 Sample preparation

Experiments were carried out in glassware. To minimize losses of BPA onto glass surfaces, all glassware was cleaned as follows. Briefly, glassware was heated in an oven at 200 °C to remove any water adsorbed on the glass surfaces, and the heated glassware was soaked overnight in a 5 % trimethylchlorosilane solution in toluene.

The glassware was rinsed with toluene and methanol and then dried in an oven at 100 °C for 1 h. Analysis of the samples (with sugar and without sugar) was carried out on an hourly interval from the first hour of purchase of the samples. The fifth samples (with sugar and without sugar) were analyzed 24 h after purchase of the samples. The control samples were analyzed 1 h after purchase of the samples.

3.4 Determination of Bisphenol A

3.4.1 Extraction and clean-up

Extraction and clean-up was based on QuEChERS as proposed by UCT, (2013). The sample (porridge) was poured into a glass beaker and homogenized using IKA® homogenizer for 10 min at high speed. A weight of 10 ± 0.05 g of the porridge was weighed into a 50 mL centrifuge tube and 10 mL of acetonitrile added. The tube was shaken vigorously at 400 rpm for 10 min. Subsequently 4 ± 0.05 g of $MgSO_4$ and 1 ± 0.05 g of NaCl were added and the tube was vortex for 10 sec to break up salt agglomerates. The tube was shaken at 400 rpm for 10 min and centrifuged at 4000 rpm for 5 min. The

supernatant was dried at 40 °C, reconstituted in 2 mL of mobile phase solution and 100 uL was injected into the HPLC.

3.4.2 HPLC determination

Stock solutions of BPA (Fluka Analytical) were prepared in acetonitrile at final concentration of 1 ug/mL. Working standards were made by serial dilution from stock and calibration standards of 5 – 30 ng/g were prepared accordingly.

HPLC analysis was based on Lateef (2011) with modification. A Cecil-Adept Binary Pump HPLC coupled with Shimadzu 10 AxL fluorescence detector (Ex: 275 nm, Em: 313) with Supelco Discovery C18, 5 µm, 4.6 x 10 mm was used for analysis. Mobile Phase composition was water/methanol (45:65, v/v) at 0.9 ml/min with column temperature maintained at 40 ° C.

3.5 Data analysis

The @Risk software was used to fit the distribution of the hazard concentration (HC), consumption rate (CR), exposure frequency/duration (EFD), body weight (BW), averaging time (AT) and the risk estimated using the empirical models:

$$CDI = \frac{C \times CR \times EFD}{BW \times AT}$$

Where; C = concentration of Bisphenol A (mg/g), CR = contact rate (mL/day), EF = exposure frequency (days/year), ED= exposure duration (years), BW = body weight (kg), AT = average time of exposure (days).

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Risk parameters	Data sources and values	Fitted distribution/references
Hazard concentration (HC)	Dietary Recall Food Survey	
✓ “Koko” with sugar		Triang(0.00011001,0.00011001,0.0020459)
✓ “Koko” without sugar		Expon(6.93922e-005,Riskshift(-1.39443e-006))
Consumption rate (HC)	Dietary Recall Food Survey	
✓ “Koko” with sugar		Geomet(0.0017020)
✓ “Koko” without sugar		NegBin(8,0.0092064)
Exposure frequency	300-365 days per year	Uniform(300,365)
Exposure duration	5 -70 years	Uniform(5,70)
Body weight	Dietary Recall Food Survey	
✓ “Koko” with sugar		NegBin(22,0.26773)
✓ “Koko” without sugar		NegBin(22,0.26928)
Averaging time (AT)	365 x 70 years	

Reference dose (RfD)

0.00005mg/g-day

US EPA, 2010

$$\text{Hazard Quotient (HQ)} = \frac{CDI}{RfD}$$

Table 3.1: Model parameters and primary and secondary data sources used for the estimation of risk



The data was tabulated in Microsoft Excel and analyzed using Palisade @Risk software to simulate BPA risk in “Hausa koko” packaged in transparent plastic film using the Monte Carlo simulation. The data was entered in Excel spreadsheet columns as hazard concentration, consumption frequency, volume of medium consumed, exposure frequency, exposure duration, averaging time and body weight. Each column of data was fitted to an appropriate or best fit distribution on the basis of Akaike Information Criterion (AIC) using the @Risk “distribution fitting” function specifying whether it is discrete or continuous and writing the aggregate output to a designated cell. Each value so generated was added to a unit excel cell using the “add output” tool and simulated at first order Monte Carlo simulation at ten thousand iterations. A construct of the empirical model was built using Excel formulas into which the model parameters were referenced to determine the risk using the reference dose value from IRIS (US EPA, 2010). The risk value so generated to cell was added to output and simulated at first order Monte Carlo simulation at ten thousand iterations. Excel reports, including graphs of the outputs were then extracted as results for discussion.

3.5 Statistical Analysis

In order to establish whether there was a statistical significance difference between the mean concentration of bisphenol A in “Hausa koko” with sugar and that without sugar, hypothesis testing was conducted on the level of BPA in samples with or without sugar. Using Microsoft Excel 2010, t-Test: paired two samples for means was run for the level of BPA in samples with or without sugar. The hypothesized mean was assumed to be zero and the significance level was set at 0.05 (5 %).

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 Population Description

A summary of the sociodemographic characteristics of the participants is shown in Figure 4.1 and 4.2. The highest number of participants were males representing 196 (61.3 %) whilst the least number of participants were females representing 124 (38.7 %) of the total number of 320 participants. The number of participants for the sexes was not the same since participants were randomly recruited for the study.

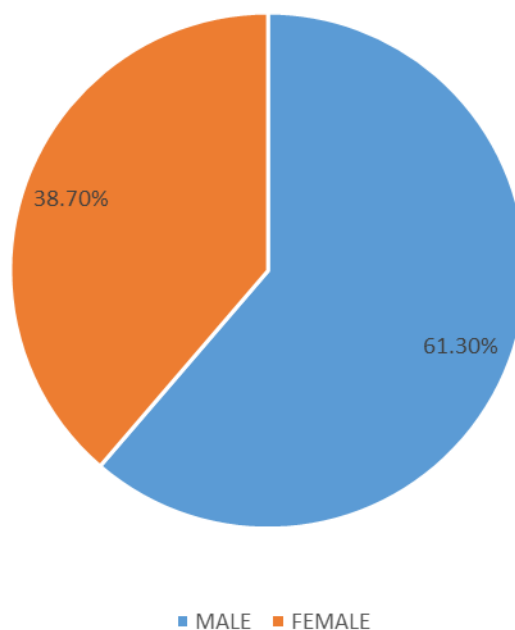


Figure. 4.1: The sex of consumers used in the study

Consumers between the ages of 21 and 30 years were seen to consume the product more frequently. They represented 39.4 % of the total respondents. The youngest of the consumers were between the ages of 0 and 10 years. The results show that most consumers of “Hausa koko” within the study area were the youth.

45.00%

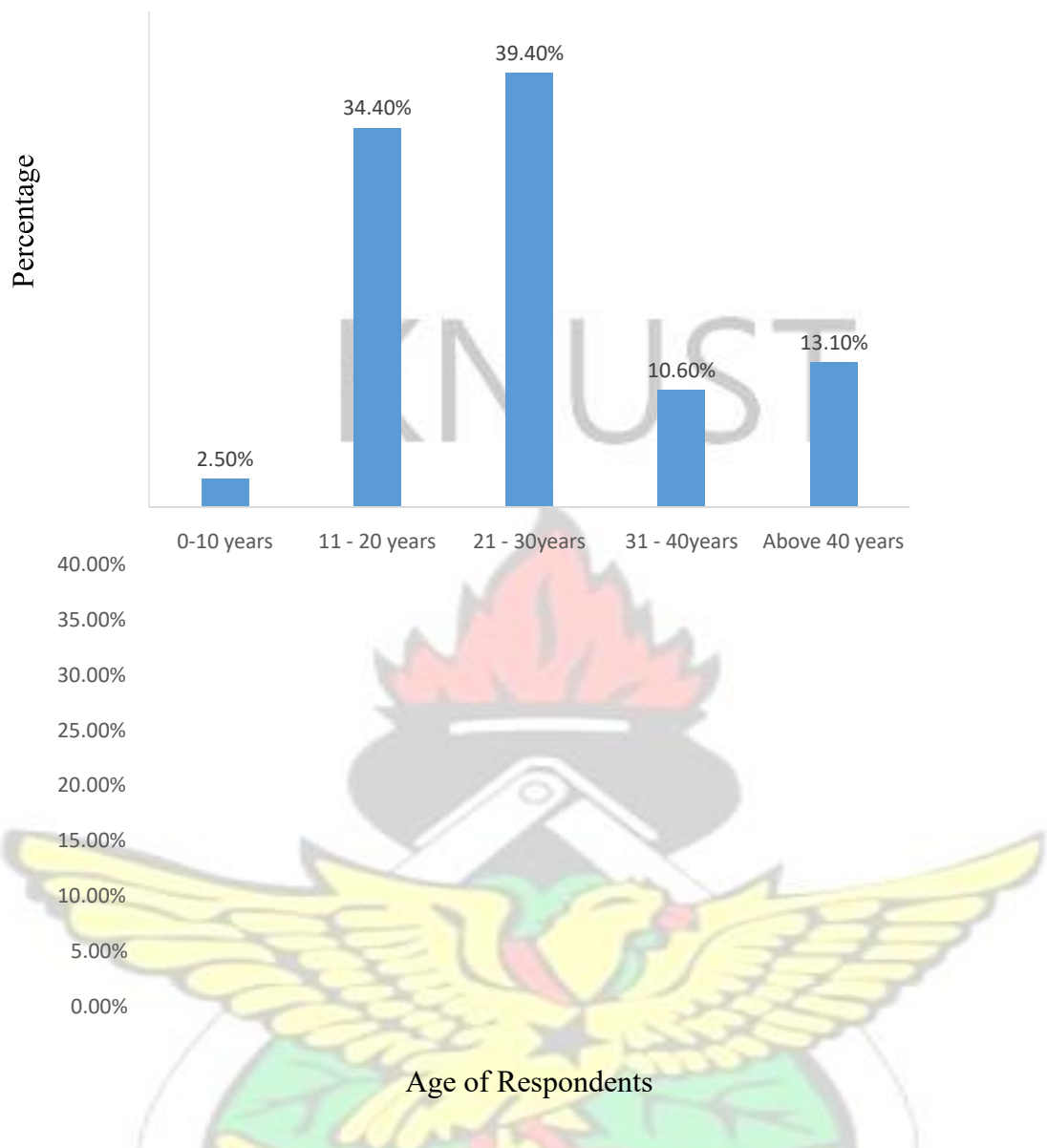


Figure 4.2 The age (yr) of consumers used in the study

4.2 Exposure factors

Since the exposure factors are used to derive CDI and hazard quotient, the research sought to get information about the following exposure factors; the number of times the respondents consume “Hausa koko” in a day, the volume of “Hausa koko” consumed by the respondents in mL, the concentration of BPA in “Hausa koko” packaged in transparent plastic film and the body weight of the respondents.

4.2.1 Frequency of consumption of “Hausa koko”

From figure 4.3 the mean daily consumption of “Hausa koko” in transparent plastic film was 1.08. The modal daily consumption of “Hausa koko” was 1.00. The number of times of consumption of “Hausa koko” by the lowest exposure group (5th percentile) of “Hausa koko” within the study area was 0.00 per day. That of the highest exposure group (95th percentile) of the population was 2.00 per day. ‘Hausa koko’ is normally consumed with sugar, it was therefore not surprising that 93.8 % of the respondents consumed ‘Hausa koko’ with sugar. Only 6.2 % consumed it without sugar. This according to the consumers was due to health issues.

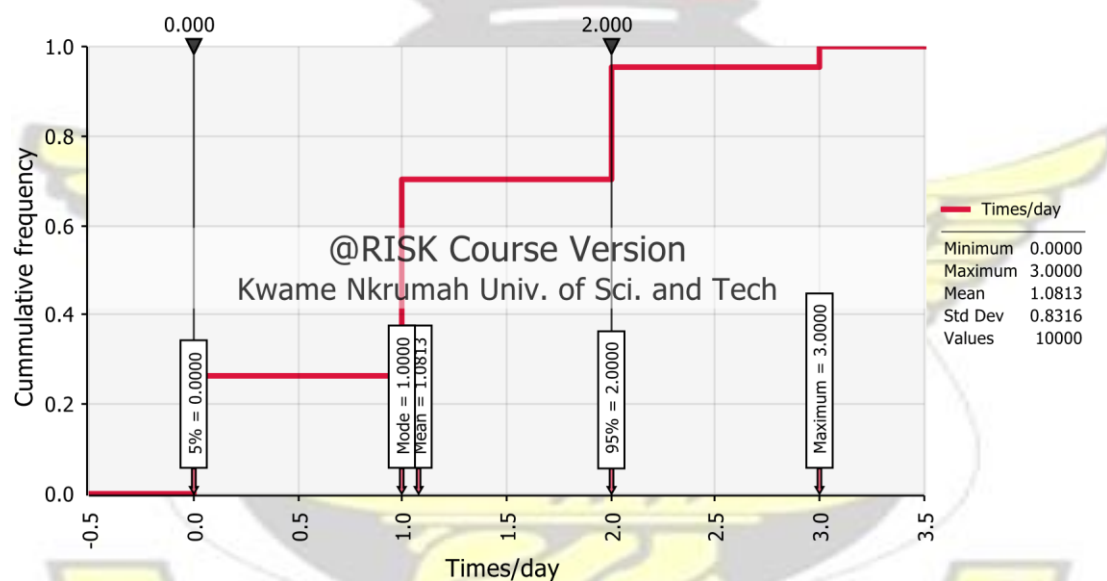


Figure 4.3: The number of times (per day) of consumption of “Hausa koko” by consumers used in the study

4.2.2 Volume of “Hausa koko” consumption

From the analysis of the surveyed food frequency questionnaire (fig 4.4), the mean, median and modal volumes in mL of “Hausa koko” consumed by the population under study were 538.50, 533.00, and 505.00, respectively. The minimum and maximum

volumes in mL of “Hausa koko” consumed by the participants were 239.00 and 1,021.00 respectively. The volume of “Hausa koko” in mL consumed by the lowest exposure group (5th percentile) within the population was 386.00 and that for the highest exposure group (95th percentile) was 712.00.

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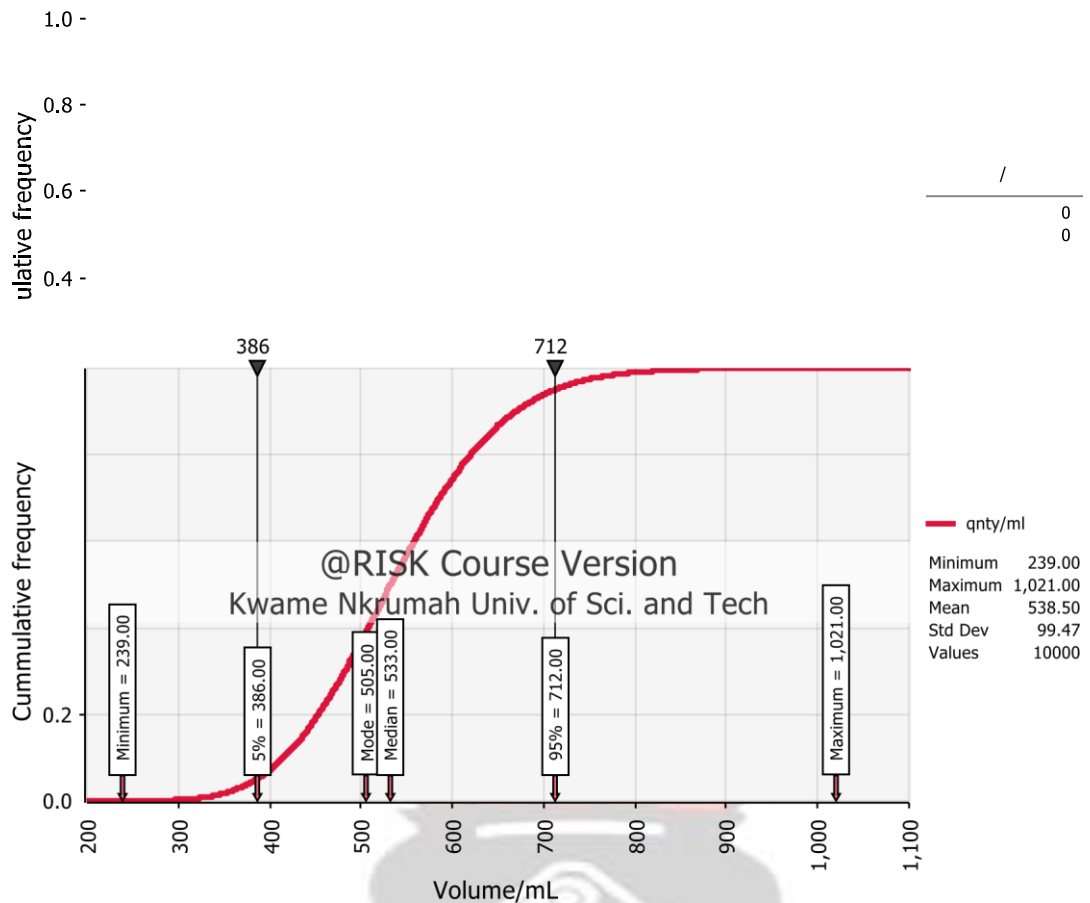


Figure 4.4: The volume (mL) of “Hausa koko” consumed by consumers used in the study.

4.2.3 Concentration of BPA in “Hausa koko”

From figure 4.5, the mean concentration of BPA in “Hausa koko packaged in transparent plastic film was 3.5E-007 mg/g. The minimum and maximum levels of BPA were -5.3E-009 mg/g and 3.8E-005 mg/g, respectively. The concentration of BPA in “Hausa koko” consumed by the highest and lowest exposure group within the population (95th and 5th percentile respectively) was 1.5E-006 and -8.8E-010, respectively.



Fasano *et al.*, 2012 found maximum concentration of Bisphenol A in plastics to be 0.19 $\mu\text{g/L}$ (0.00019 mg/g). Le *et al.* (2008) and Cao *et al.* (2008) however found a maximum migration of Bisphenol A in PC bottles to be 7.67 $\mu\text{g/L}$ (0.00767mg/g). The difference in the concentrations could be as a result of the amount of BP A used in the manufacturing of the different products.

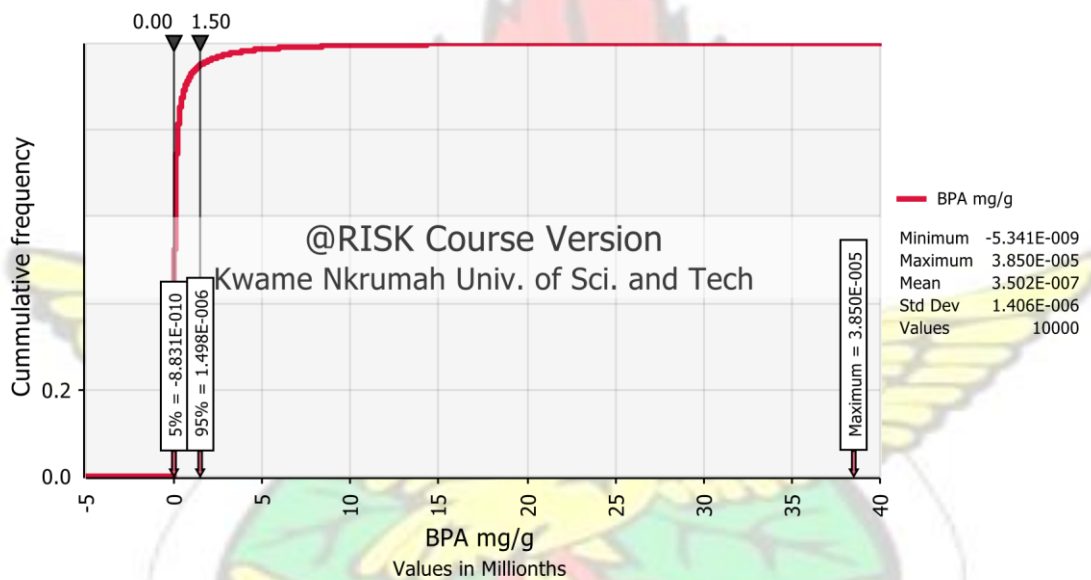


Figure 4.5: The concentration (mg/g) of BPA used in the study

4.2.4 Body weight of respondents

From the analysis of the surveyed data (figure 4.6), the 95th percentile body weight of consumers of “Hausa koko” in transparent plastic film within the study area was 86.00 kg. The analysis also indicated that the 5th percentile body weight was 37.00 kg. The



mean, median and modal body weight of the respondents was 60.13 kg, 59.00 kg and 56.00 kg, respectively. The minimum body weight of the respondents was 16.00 kg and the maximum body weight was 131.00 kg. The WHO (2007) reports body weights of healthy individuals to be between 28 kg to 89 kg for men and women based on height. Results from this study fall in line with body weights provided by the WHO which implies that the individuals used were healthy.

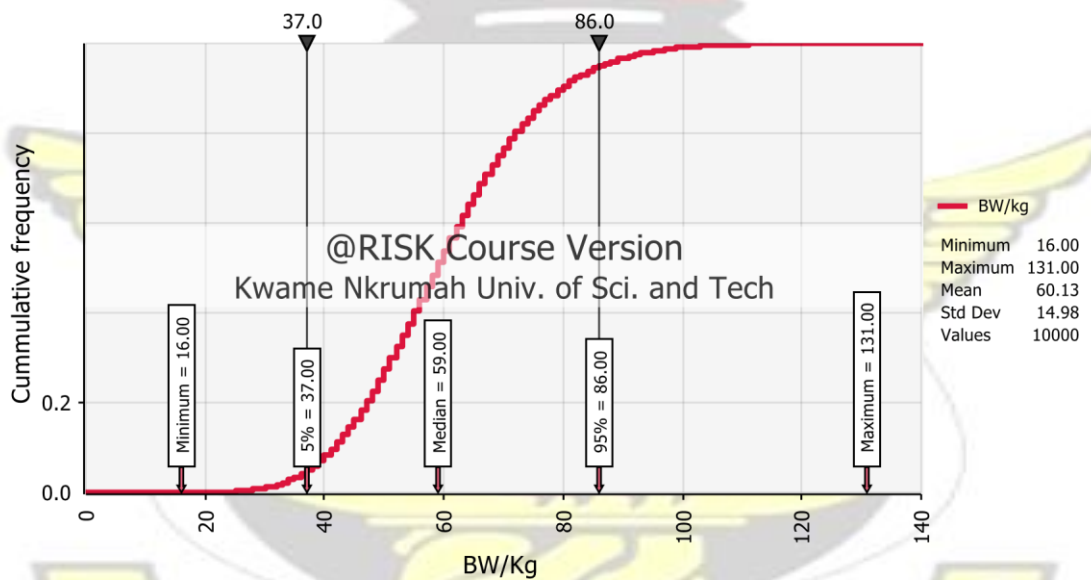


Figure 4.6: The body weight (kg) of consumers used in the study

1.0 -
0.8 -
0.6 -
0.4 -
relative frequency

1
0
0

4.3 Statistical Results

4.3.1 Effect of storage time on the concentration of BPA in “Hausa koko” The concentration of bisphenol A in ‘Hausa koko’ in transparent plastic films was evaluated with storage time. All the control samples did not have any concentration of bisphenol A in the products. For all samples, the concentration of bisphenol A increased with increase in storage time, which is the period between time of purchase and that of the analysis of “Hausa koko” samples. The concentrations however, varied for the different vendors. This may be due to the fact that the various vendors used plastic films produced from different companies to package their products. The concentrations varied from below the limit of detection (<0.005 ng/ml) to 1.92 ng/ml (Figure 4.7) for the 5 vendors.

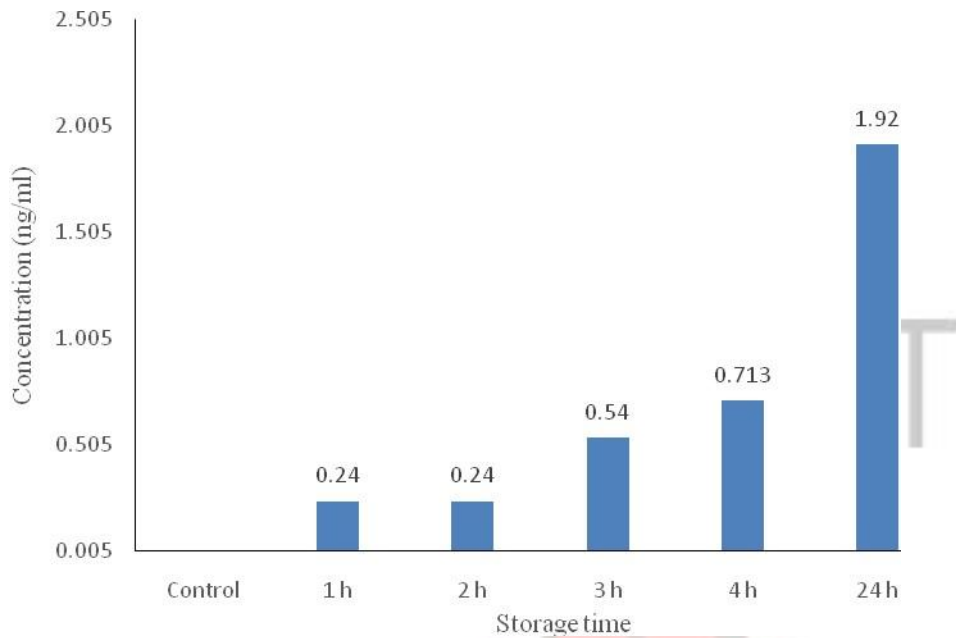


Figure 4.7: Changes in concentration of BPA with storage time (hr)

The results obtained support the findings of Le *et al.* (2008), who found that the concentration of BPA released from polycarbonate bottles increased over time into water at room temperature. In a study carried out by Howdeshell *et al.* (2003) involving polycarbonate animal cages, the team of researchers made similar observations about the migration of BPA into water at room temperature.

4.3.2 Effect of sugar on the concentration of BPA in “Hausa koko”

Figure 4.8 gives results of the concentrations of Bisphenol A found in ‘Hausa koko’ with and without sugar. Concentrations of Bisphenol A in ‘Hausa koko’ with sugar ranged from <0.005 ng/ml) to 1.92 ng/ml.

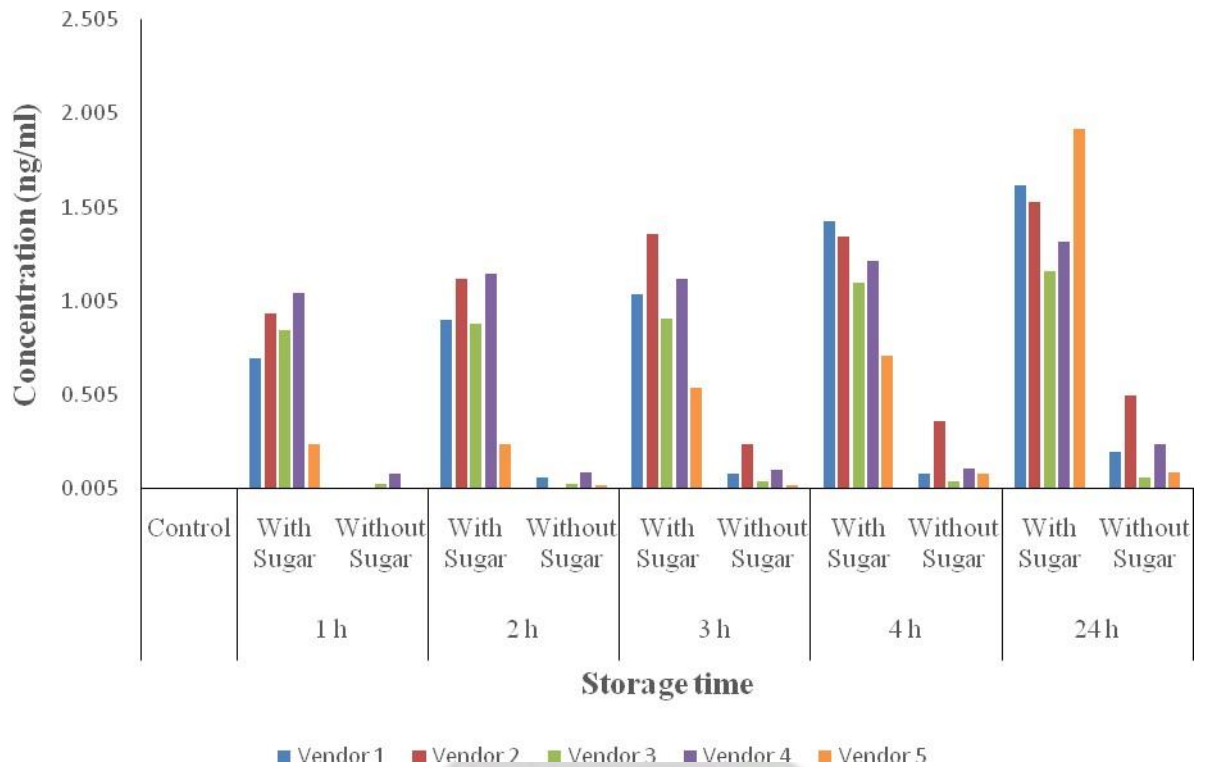


Fig 4.8: Effect of sugar on concentration of BPA in ‘Hausa koko’

The various concentrations of BPA in “Hausa koko” without sugar ranged from <0.005 ng/ml to 0.5 ng/ml. The values obtained were however lower than those with sugar in them. The results obtained are in agreement with work by Sungur *et al.* (2014) into foods packaged in containers whose inner surface had been lined with plastic film displayed for sale on the Turkish market. The researchers concluded that the amount of BPA increases with an increase in the amount of glucose and NaCl.

4.3.3 Effect of temperature on concentration of BPA in “Hausa koko”

The study recorded BPA concentration in the porridge at two different temperatures. Thus, 60 °C – 70 °C during the time of purchase on the first visit and 40 °C – 5 0°C at the time of purchase on the second visit. The concentrations of BPA decreased with a decrease in temperature and vice versa (Fig. 4.9).

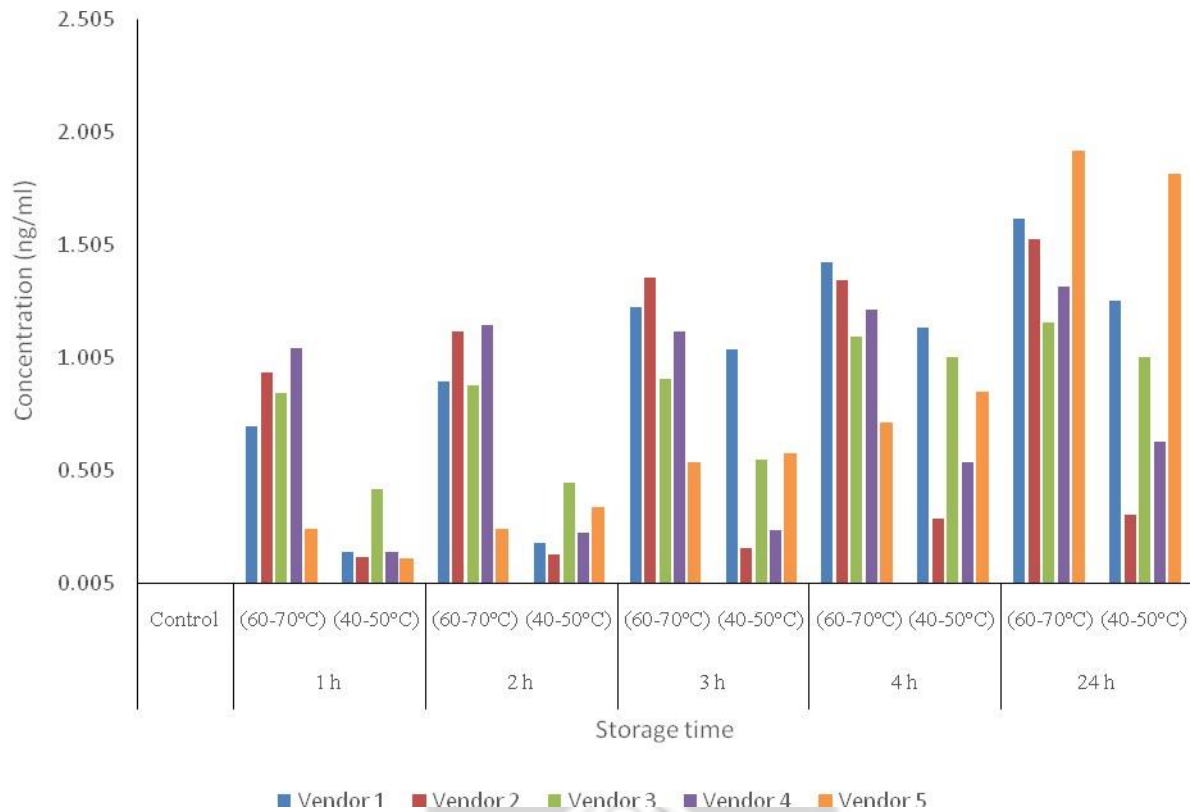


Figure 4.9 Effect of temperature on concentration of BPA in “Hausa koko”

Various studies have reported the effects of temperature on migration of BPA.

Exposure to the monomer has been reported to occur as a result of the migration of BPA out of these products when they are exposed to high heat. Krishnan *et al.* (1993) reported that polycarbonate flasks leached BPA into media in the autoclaving process. The results obtained are in agreement with work by Le *et al.* (2008). The researchers focused on the relationship between BPA migrations into water at room temperature versus the migration into hot or boiling water (Le *et al.*, 2008). Their study showed that BPA was leached from all polycarbonate bottles. It was found that the concentration of BPA released from the polycarbonate bottles increased over time into water at room temperature. Researchers found that the concentration of BPA in heated samples was twice that of samples at room temperature (Le *et al.*, 2008).

4.3.4 Effect of pH on BPA concentration in “Hausa koko”

The release of BPA into food matrix can be through leakage from plastic packaging. This leakage is caused by acidic or basic conditions which accelerate the hydrolysis of the ester bonds linking the plastic polymer therefore releasing monomers of BPA into the product in contact with the plastic material (Richter *et al.*, 2007). Results obtained from this current study showed that the migration of Bisphenol A was higher in “Hausa koko” having a pH of 3.4 than those at a pH of 4.1.

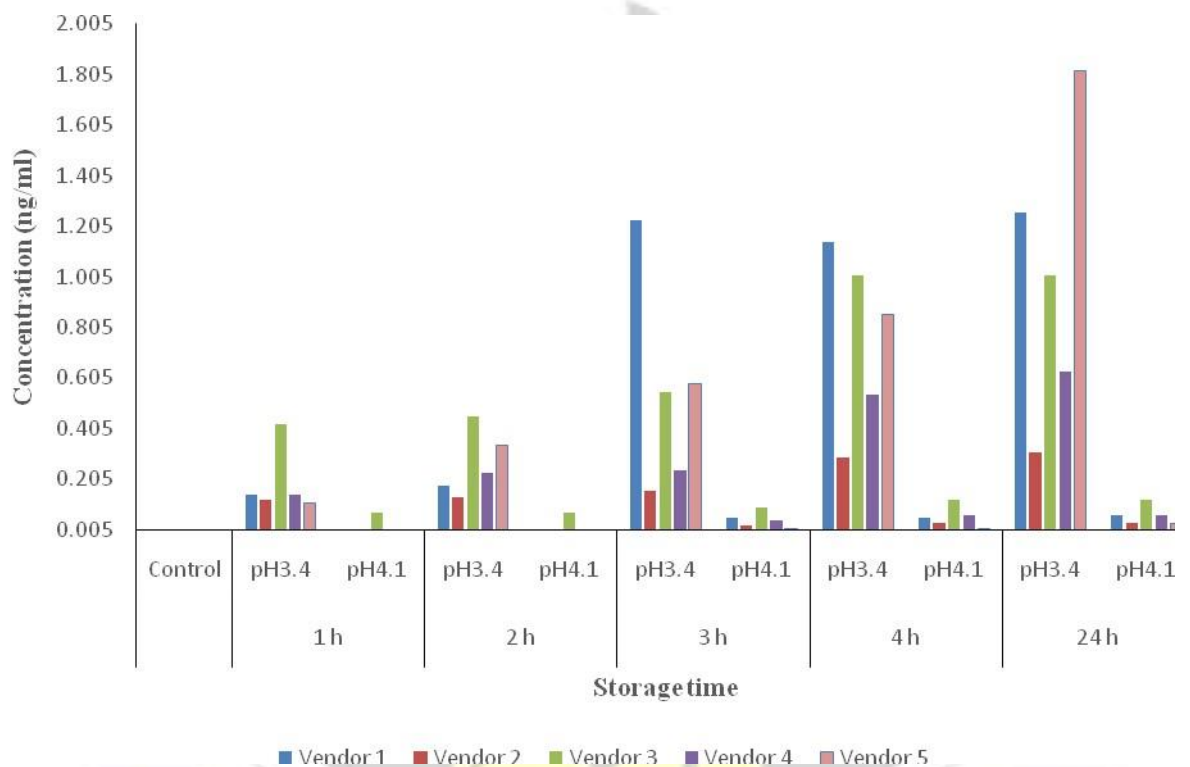


Figure 4.10: Effect of pH on BPA concentration in “Hausa koko”

4.4 Significance testing.

The p-value obtained from the test was 0.00. Since this value is less than the significance level (0.05), there is a significant difference between the mean concentration of BPA in “Hausa koko” with sugar and that without sugar. In view of this the data obtained from the questionnaire were grouped into two categories, ie

“Hausa Koko with sugar and “Hausa koko” without sugar. The risk (hazard quotient) was then calculated for each of the two categories.

Table 4.1: Summary results of t-Test for the two sample means

	<i>With Sugar</i>	<i>Without Sugar</i>
Mean	0.00	0.00
Variance	0.00	0.00
Observations	50	50
Pearson Correlation	0.60	
Hypothesized Mean Difference	0	
Df	49	
t Stat	11.65	
P(T<=t) one-tail	0.00	
t Critical one-tail	1.68	
P(T<=t) two-tail	0.00	
<u>t Critical two-tail</u>	<u>2.01</u>	

4.5 Hazard quotient

The Chronic Daily Intake (CDI) obtained as defined under materials and methods was divided by a reference dose (R_fD) of 0.00005 mg/g (USEPA, 2010) to obtain the hazard quotient.

For a population to be at a significant risk, the hazard quotient should be above 1. However, if the value is below 1 it can be concluded that there is no significant risk or no adverse health effects is expected. From the study conducted (Table 4.2), the mean, median and modal hazard quotient for “Hausa koko” without sugar consumed by both males and females were 0.00, 0.00 and -0.00, respectively. The hazard quotient for

highest and lowest exposure group within this group was 0.01 and 0.00, respectively. Since these figures are less than 1, consumers of “Hausa koko” (both male and female) without sugar within the study area are at no significant risk.

Table 4.2: Summary statistics for risk (HQ)

<i>Sample</i>	<i>Mean</i>	<i>Median/50th Percentile</i>	<i>mode</i>	<i>5th Percentile</i>	<i>95th Percentile</i>
<i>with sugar (males and females)</i>	0.01	0.00	0.00	0.00	0.04
<i>with sugar (males only)</i>	0.01	0.01	0.00	0.00	0.04
<i>with sugar (Females only)</i>	0.01	0.00	0.00	0.00	0.04
<i>without sugar (males and females)</i>	0.00	0.00	-0.00	0.00	0.01
<i>without sugar (males only)</i>	0.00	0.00	0.00	0.00	0.01
<i>without sugar females only)</i>	0.00	0.00	0.00	0.00	0.00

From the analysis of the surveyed data (Table 4.2, figure 4.11 and figure 4.12), consumption of “Hausa koko” with sugar poses no significant health risk to the population under study. This is because the mean, median, mode, 5th and 95th percentile hazard quotient (0.01, 0.00, 0.00, 0.00 and 0.04, respectively) were less than 1.

Again, the hazard quotient for males and females within the population under study for ‘Hausa koko’ with or without sugar was determined. From Table 4.2, it was observed that consumption of “Hausa koko” with sugar by males only and females only within the study area poses no significant health risk since the hazard quotient for the mean, median/50th percentile, mode, 5th and 95th percentiles was less than 1.

The observation made for “Hausa koko” without sugar consumed by males only and that of females only within the population under study was no different. From Table 4.2 the hazard quotient for the lowest exposure group (males only and females only) for “Hausa koko” without sugar was 0.00 and 0.00, respectively. That of the highest exposure group for males and females consuming “Hausa koko” without sugar was 0.01 and 0.00, respectively. Moreover, the mean, median and modal hazard quotient for consumers of “Hausa koko” without sugar (males only and females only) was 0.00, 0.00, 0.00 and 0.00, 0.00, 0.00, respectively. Since these figures are less than 1, consumption of “Hausa koko” without sugar by either males only or females only poses no significant health risk to the population under study.

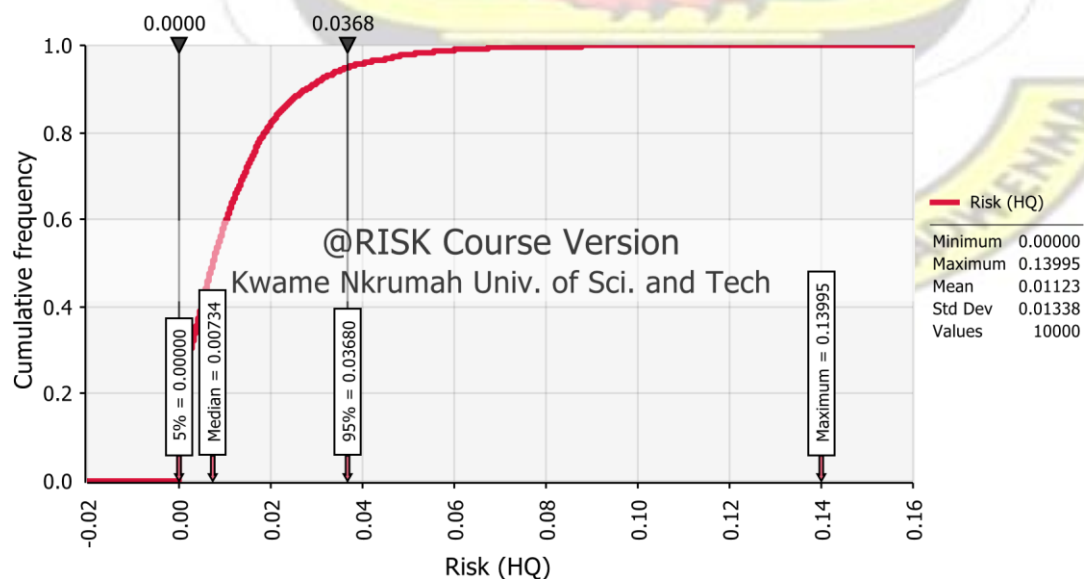


Figure 4.11: Risk (hazard quotient) for “Hausa koko” with sugar

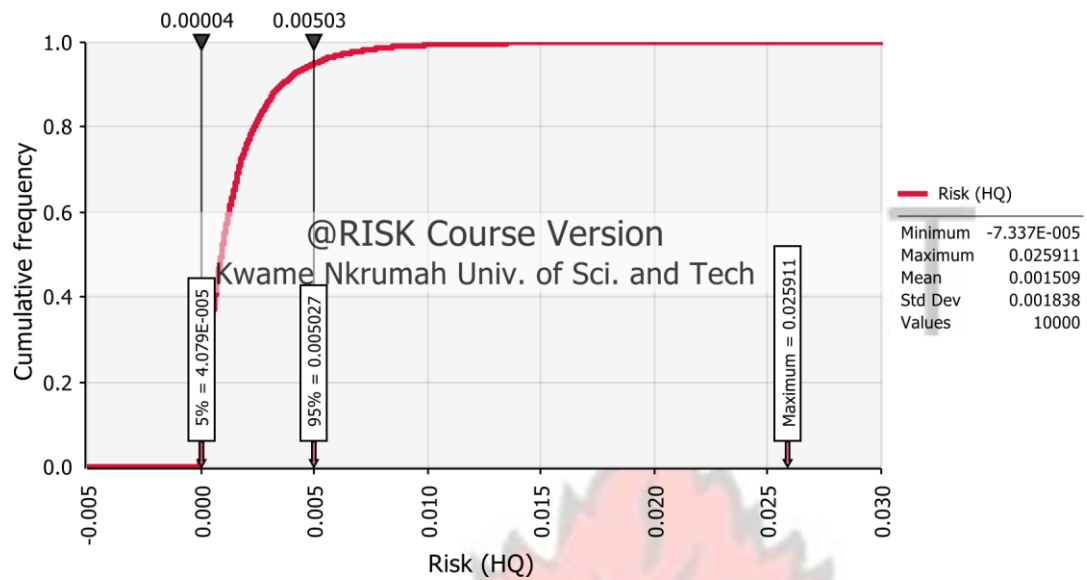


Figure 4.12: Risk (hazard quotient) for “Hausa koko” without sugar

4.6 Sensitivity Analysis

Correlation coefficient is a statistical measure of the strength of relationship between two variables. Variables which exhibit this feature are considered to depend on one another in some way. Values between +1 and -1 are used to connote correlation coefficient. A coefficient of +1 indicates a perfect positive correlation. That is, an increase or decrease in the value of one variable will result in an increase or decrease in the value of the other variable. A coefficient of -1 indicates a perfect negative correlation. That is an increase in the value of one variable will result in a decrease in the value of the other variable and vice versa. It could be observed from Fig 4.13 that the number of times of consumption had a strong positive relationship with the hazard quotient. It was also observed that the concentration of BPA and the quantity of ‘Hausa koko’ with sugar had a moderate positive relationship and extremely weak positive relationship respectively with the hazard quotient. Contrary, it was observed that the

body weight of the participants had an extremely weak negative relationship with the hazard quotient. This is because, from the equation 1 used to derive CDI, body weight is inversely proportional to CDI.

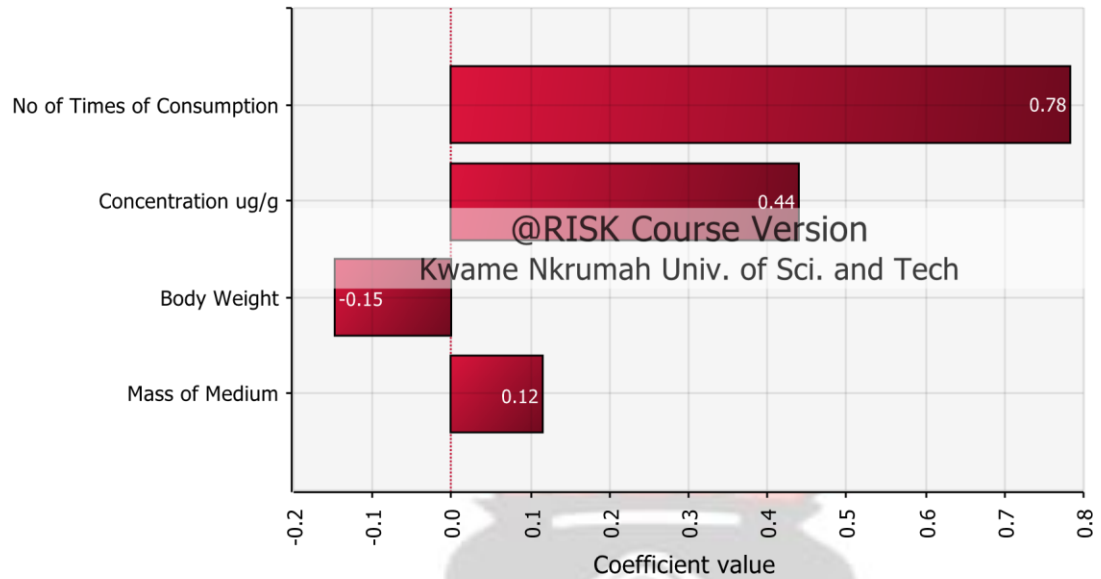


Figure 4.13: Correlation coefficient for ‘Hausa koko’ with sugar

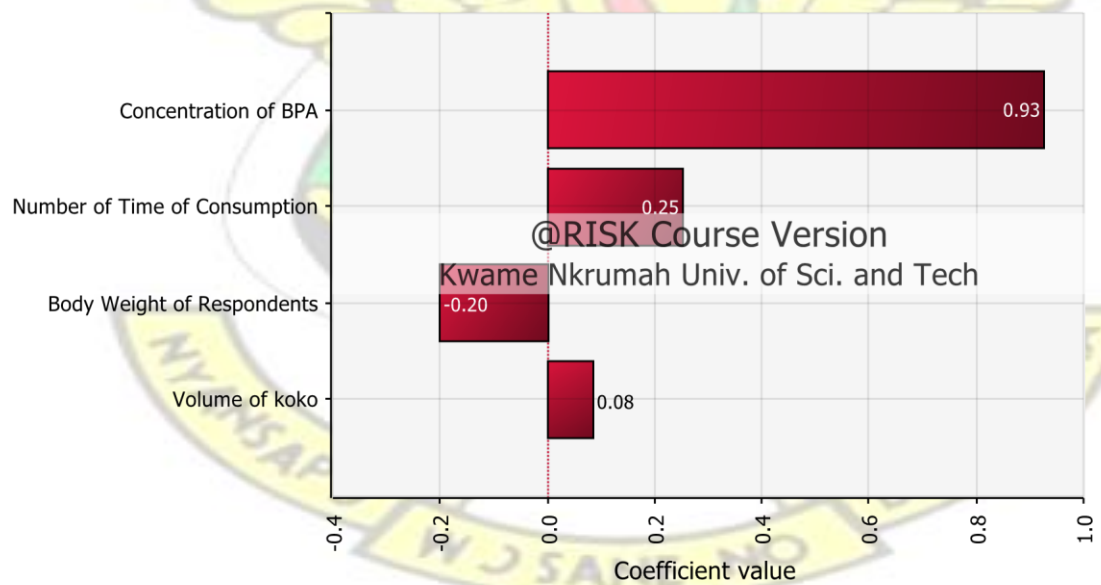


Figure 4.14 Correlation coefficient for ‘Hausa koko’ without sugar

Fig 4.14 indicated a very strong positive relationship between the concentration of BPA in 'Hausa koko' without sugar and the hazard quotient. A weak positive relationship was observed for the number of times of consumption of 'Hausa koko' without sugar and the hazard quotient. Again, a none to extremely weak positive relationship was observed between the volume of 'Hausa koko' without sugar and the hazard quotient. However, it was observed that the relationship between the hazard quotient and body weight of participants was extremely weak negatively, since body weight is inversely proportional to CDI.



CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The study concludes that the concentrations of bisphenol A in “Hausa koko” packaged in transparent plastic film increased with increase in storage time, pH, temperature and sugar levels. Again the concentrations of bisphenol A in “Hausa koko” with or without sugar ranged from <0.005 ng/ml to 1.9 ng/ml.

The study further concludes that consumers of “Hausa koko” with or without sugar in transparent plastic film within the study area are at no significant risk. Moreover, consumption of “Hausa koko” with or without sugar in transparent plastic film by male consumers only and female consumers only within the study area poses no significant health risk.

5.2 Recommendation

It is recommended that the study should be replicated nationwide so it can be ascertained whether the entire Ghanaian population is at risk or not. The percentage of BPA used in the formulation of plastics should also be determined by future studies so the percentage that migrates into food can be quantified or determined.

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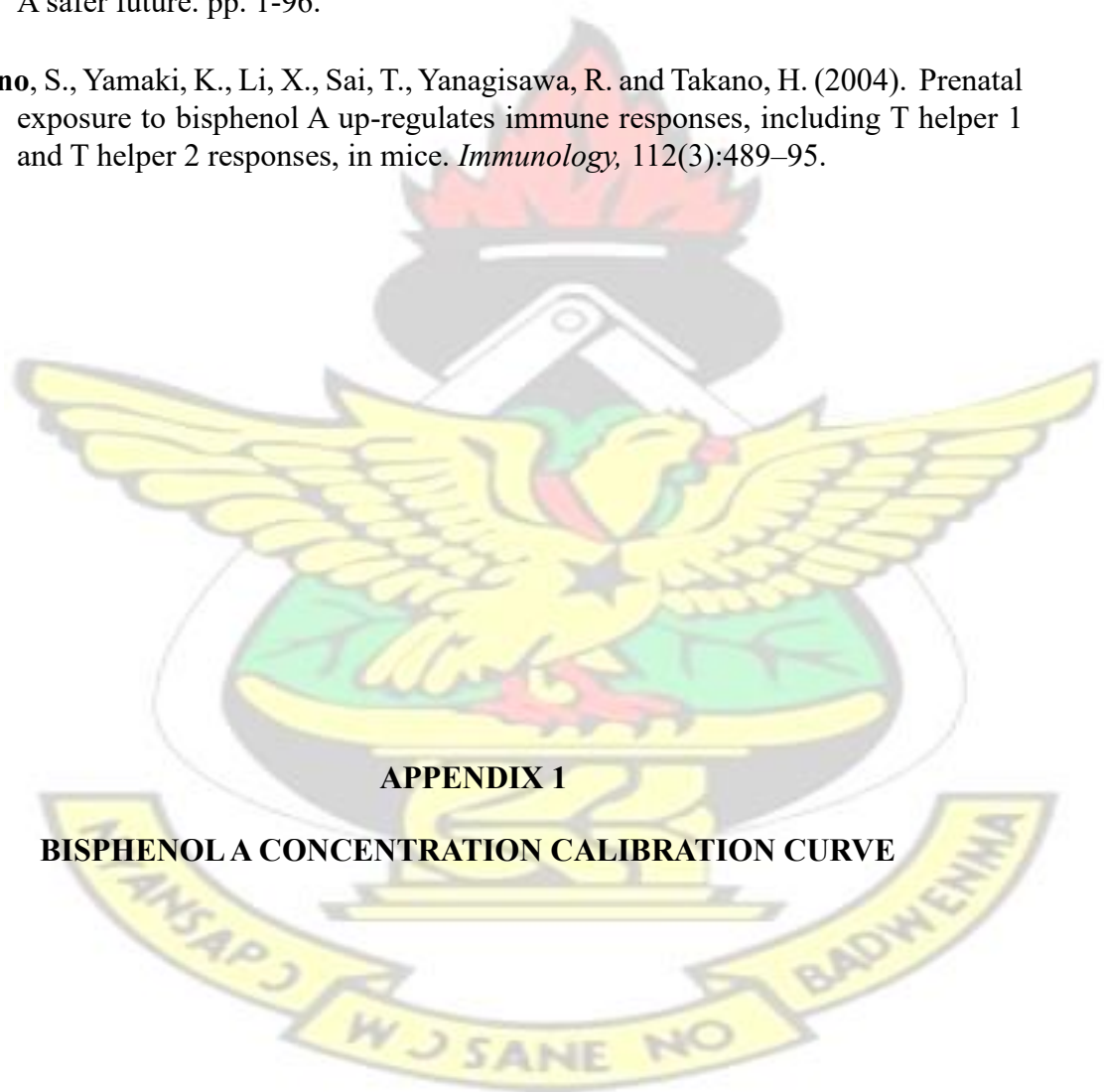
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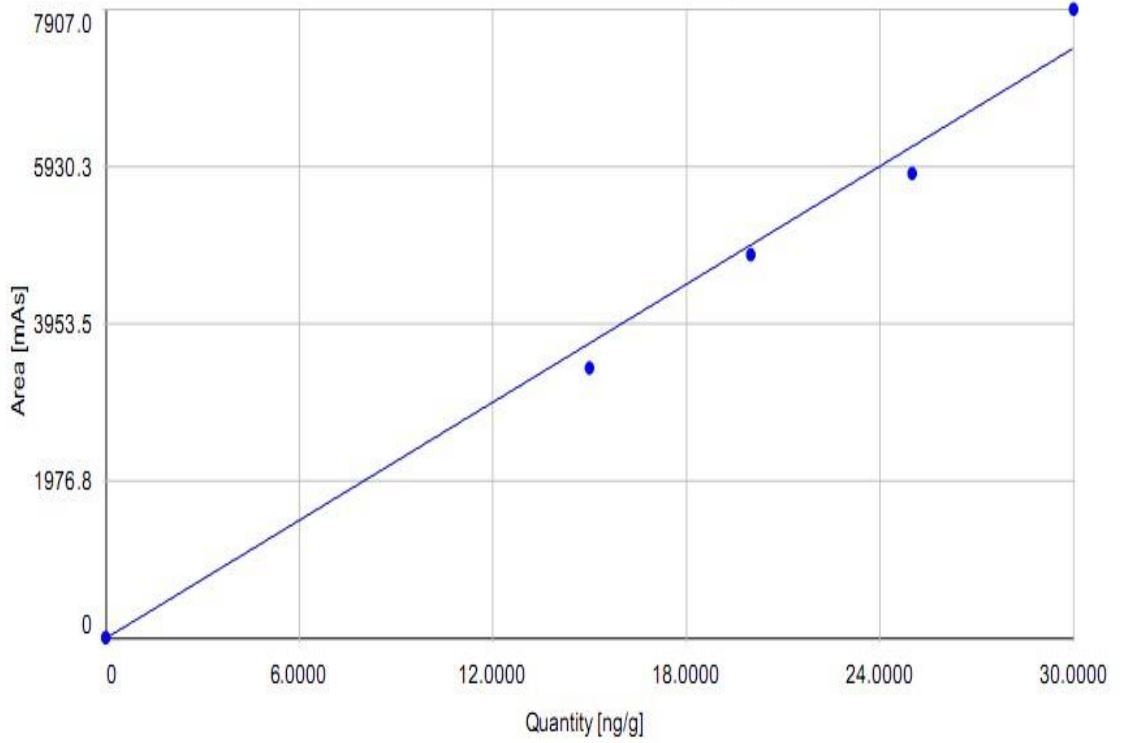
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APPENDIX 1

BISPHENOL A CONCENTRATION CALIBRATION CURVE

031115_BPA_Agilent_Waters Column_275_313 (\2015 Mycotoxin Lab\) - 45C685A4F9BD7660v1
 Component - BPA
 Quantity [ng/g] = 0 + 4.04009 * Area [As]
 r2 = 0.985295



APPENDIX 2

SAMPLE (WITH SUGAR) BISPHENOL A CONCENTRATION (ng/g)

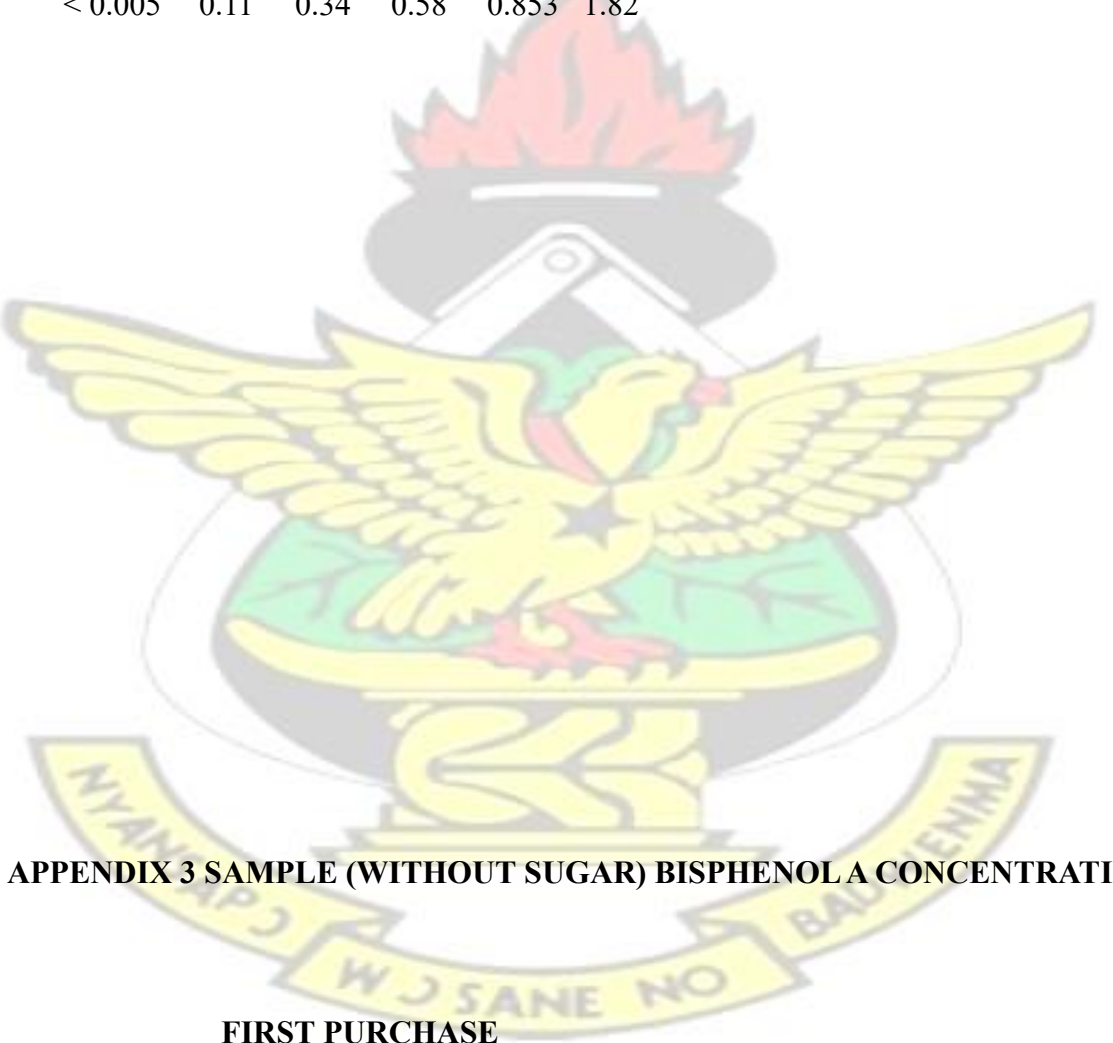
FIRST PURCHASE

Vendor	Control	1hr	2hrs	3hrs	4hrs	24hrs
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A	< 0.005	0.7	0.9	1.04	1.43	1.62
B	< 0.005	0.94	1.12	1.36	1.35	1.53
C	< 0.005	0.85	0.88	0.91	1.1	1.16
D	< 0.005	1.05	1.15	1.12	1.22	1.32
E	< 0.005	0.24	0.24	0.54	0.713	1.92

SECOND PURCHASE

Vendor	Control	1hr	2hrs	3hrs	4hrs	24hrs
A	< 0.005	0.14	0.18	1.23	1.14	1.26
B	< 0.005	0.12	0.13	0.16	0.29	0.31
C	< 0.005	0.42	0.45	0.55	1.01	1.01
D	< 0.005	0.14	0.23	0.24	0.54	0.63
E	< 0.005	0.11	0.34	0.58	0.853	1.82



APPENDIX 3 SAMPLE (WITHOUT SUGAR) BISPHENOL A CONCENTRATION (ng/g)

FIRST PURCHASE

Vendor	Control	1hr	2hrs	3hrs	4hrs	24hrs
A	< 0.005	< 0.005	0.06	0.08	0.08	0.2
B	< 0.005	< 0.005	< 0.005	0.24	0.36	0.5
C	< 0.005	0.03	0.03	0.04	0.04	0.06
D	< 0.005	0.08	0.09	0.1	0.11	0.24

E < 0.005 < 0.005 0.02 0.02 0.08 0.09

SECOND PURCHASE

Vendor Control	1hr	2hrs	3hrs	4hrs	24hrs	
A	< 0.005	< 0.005	< 0.005	0.05	0.05	0.06
B	< 0.005	< 0.005	< 0.005	0.02	0.03	0.03
C	< 0.005	0.07	0.07	0.09	0.12	0.12
D	< 0.005	< 0.005	< 0.005	0.04	0.06	0.06
E	< 0.005	< 0.005	< 0.005	0.01	0.01	0.03



APPENDIX 3 DESCRIPTIVES

Weight of respondents taken by researcher in Kilograms

BW/kg	Frequency	Percent
< 15	1	0.3
15 – 30	6	1.9
31 – 46	50	15.6
47 -62	126	39.4

63 – 78	103	32.2
79 – 94	32	10
95+	2	0.6
Total	320	100.0

KNUST

Sex and age of respondents in years

Sex	Frequency	Percent
Male	196	61.3
Female	124	38.7
Total	320	100.0

Age/yrs	Frequency	Percent
< 10	8	2.5
11 – 20	110	34.4
21 – 30	126	39.4
31 – 40	34	10.6
40+	42	13.1
Total	320	100.0

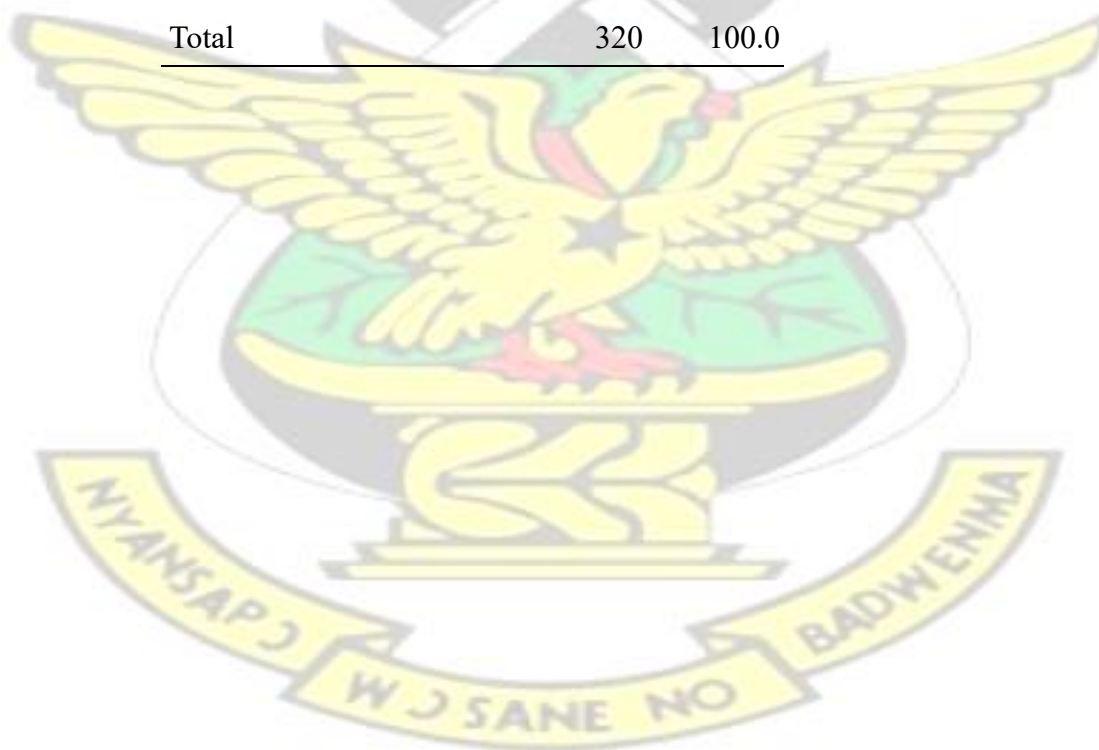
Employment status and level of education

Employment status	Frequency	Percent
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Employed	168	52.5
Unemployed	54	16.9
Student	98	30.6
Total	320	100.0

KNUST

Education	Frequency	Percent
Never been to formal school	12	3.8
Primary	25	7.8
Junior High School	63	19.7
Senior High School	133	41.6
Tertiary	87	27.1
Total	320	100.0



Consumption of “Hausa koko with/without sugar by respondents

“Hausa koko	Frequency	Percent
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Sugar	300	93.8
Without sugar	20	6.2
Total	320	100.0

KNUST

Time (mins) of consumption of “Hausa koko after purchase by respondents

Time/mins	Frequency	Percent
< 30	302	94.4
31 – 60	8	2.5
60+	10	3.1
Total	320	100.0



APPENDIX 4 QUESTIONNAIRE

Please tick where applicable

1. Sex Male () Female ()
2. Age (yrs)
3. Employment background? Student () Employed () Unemployed ()
4. Highest level of education
5. Occupation if employed
6. Do you consume 'hausu koko' packaged in transparent plastic film?
 Yes () No ()
7. How do you prefer your 'hausu koko'?
 With sugar () without sugar ()
8. How long do you keep 'hausu koko' packaged in transparent plastic film before consumption?
9. How much do you buy for consumption per day?

10. How many times do you consume 'hausu koko' packaged in transparent plastic film per day?
11. Have you ever left some over night? Yes () No ()
12. How did you heat it?

13. Body weight of participant (kg)
