SELENIUM AND MERCURY CONCENTRATIONS IN CANNED FISH PRODUCTS COMMONLY AVAILABLE ON THE GHANAIAN MARKET AND ESTIMATION OF THEIR SELENIUM HEALTH BENEFIT VALUES

By

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Certification

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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DEDICATION

I dedicate this work to ALMIGHTY GOD for taking me through, my lovely wife, Ms Mavis Adjei, my parents and my family.

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Glory be to God for the wonderful things He has done for me and by His grace this work has come to a successful end.

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LIST OF ABBREVIATIONS

Ag	Silver
EPA	Environmental Protection Agency
FAAS	Flame Atomic Absorption Spectrometry
GAEC	Ghana Atomic Energy Commission
GEPA	Ghana Environmental Protection Agency
GFAAS	Graphite Furnace Atomic Absorption Spectrometry
H	Hydrogen
HG-AAS	Hydride Generation Atomic Absorption Spectrometry
IAEA	International Atomic Energy Agency
FDB	Food and Drugs Board
GSA	Ghana Standard Authority
H_2Se	Hydrogen Selenide
mL min^{-1}	Milliliter per minute
D_f	Dilution factor
$Conc_{calib}$	Concentration from calibration curve
$Conc_{Se}$	Concentration of Selenium
DORM-2	Dogfish muscle
NRCC	National Research Council of Canada
CVAAS	Cold Vapour Atomic Absorption Spectrometry
ID-ICP-MS	Isotope Dilution Inductively Coupled Plasma Mass spectrometry

LIST OF ABBREVIATIONS (CONT.)

CV-AFS	Cold Vapuor Atomic Fluorescence Spectrometry
GFAAS	Graphite Furnace Atomic Absorption Spectrometry
SeHBV	Selenium Health Benefit Value
USEPA	United States Environmental Protection Agency
PEEK	Polyetheretherketone
UNEP	United Nations Environmental programme
USA	United States of America
UV	Ultra-Violet
VGA	Vapor Generation Accessory
T4	Thyroxine
T3	Triiodothyronine
MeHg	Methyl mercury
Ksp	Solubility product
HgSe	Mercury Selenide
$[CH_3Hg]_2Se$	Bis (methyl mercuric) selenide
C-Hg	Carbon Mercury bond
AAS	Atomic Absorption Spectrometry
MeHg[Cys]	Methylmercurycysteine
v/v	Volume per volume
HCl	Hydrochloric acid
w/w	Weight per weight
HNO_3	Nitric acid
NaOH	Sodium Hydroxide

LIST OF ABBREVIATIONS (CONT.)

CH_3Hg^+	Monomethylmercury
H_2O_2	Hydrogen peroxide
H_2Se	Hydrogen Selenide
Hg	Mercury
HgS	Cinnabar
$NaBH_4$	Sodium borohydride
Se	Selenium
SeO_2	Selenium (IV) oxide
w/v	Weight per volume

LIST OF SYMBOLS

I o	Incident light intensity
I t	Transmitted light intensity
a	Absorption coefficient (absorptivity)
b	Length of absorption path
c	Concentration of absorbing atoms
µg/L	Micro gram per liter
Mg Se L^{-1}	Miligram Selenium per liter
mg L^{-1}	Miligram per
µmol Se kg^{-1}	Micro mole Selenium per kilogram
µg Se g^{-1}	Micro gram Selenium per gram
µg Hg g^{-1}	Micro gram Mercury per gram
µmol Hg kg^{-1}	Micro mol Mercury per kilogram
µ	Micro
µg g^{-1}	Micro gram per gram
<	Less than
g	Gram
mg	Milligram
mg kg^{-1}	Milligram per kilogram
mg L^{-1}	Milligram per Liter
mL	Milliliter
ng g^{-1}	Nano gram per gram

LIST OF SYMBOLS (CONT.)

nm	Nano meter
ºC	Degree Celsius
mA	Milli ampere

Abstract

Canned fish products are widely and highly consumed in Ghana, due to their hygienic, delicious, and easy to consume nature. There have been numerous reports on mercury accumulation in fish, accordingly, the levels of mercury in fish and the dietary exposure to mercury through the consumption of fish have become the subject of discussion worldwide by environmental activist groups. Though the interactions between Se and Hg and their molar ratios in fish are essential factors in evaluating risks associated with dietary mercury exposure, the discussions by the environmental groups have been limited to the levels of Hg in fish alone. The study assessed the health risk [selenium health benefit value, (SeHBV)] posed by the consumption of thirteen (13) canned fish products available on the Ghanaian market. This was achieved through the determination of the concentration of Se and Hg in the fifty-two (52) blended lyophilized homogenates of fish products using hydride generation atomic absorption spectrometry (HG-AAS) and cold vapour atomic absorption spectrometry (CV-AAS). Prior to HG-AAS determination, the lyophilized fish samples were wet ashed by microwave digestion using a combination of HNO_3, HCl and H_2O_2. The digestate obtained was defatted with diethyl ether in a rotary evaporator at a water bath temperature of 45°C. Based on the concentrations of Se and Hg determined, the molar concentrations of Se and Hg ; the Se-Hg molar ratio (Se : Hg) ; the Hg-Se molar ratio (Hg : Se) ; the free Se content and hence the SeHBV were evaluated.

The validity of the HG-AAS methods for Se and Hg determinations were respectively checked by analysis of compositionally appropriate certified reference material, DORM-2 (Dogfish Muscle). Levels of Hg in the canned fish products were in the range 0.082 – 0.500 µg g^{-1}. The Se content in the fish products ranged from 0.105 to 0.875 µg g^{-1}. The molar concentrations of Hg and Se were (in ranges) 0.409 – 2.493µmol kg^{-1} and 1.330 – 11.082 µmol kg^{-1} respectively. The Se: Hg and the Hg : Se molar ratios range from 2.977 to 8.186 and 0.122 to 0.336 respectively. The free Se levels of the 13 canned fish products ranged from 0.921 to 8.589. The SeHBV ranged from 4.201 to 48.710. All the canned fish products had molar excess of Se over Hg, indicating that there was enough Se to protect against Hg toxicity. The Se:Hg molar ratio values were all greater than one (1), indicating that in all the 13 fish products the molar concentrations of Se were higher than that of Hg. The Hg:Se molar ratio values were all less than one (1), indicating that the molar concentrations of Hg were lower than those of Se. This shows that there is no Hg health risk associated with the consumption of the 13 canned fish products. The Free Se values were all positive. This indicates that there is excess Se for optimal health benefits. The SeHBV for all the canned fish products were positive consequently, the fish products are safe for human consumption.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Levels of mercury in fish have become the subject of intense campaigning by environmental activist groups, often resulting in statements about food safety [Ralston *et al.*, 2008]. Interactions between selenium and mercury and their molar ratios in fish are essential factors in evaluating risks associated with dietary mercury exposure; considering mercury content alone is inadequate [Kaneko and Nicholas, 2007]. Mercury is currently one of the most prevalent pollutants in the environment. It highly bioaccumulates through the food chain and is harmful to both humans and animals [Bierregaard *et al.,* 1999]. Selenium is an essential micronutrient with important biological and biochemical functions in organisms due to its unique antioxidant properties and its ability to regulate thyroid gland metabolism [Bierregaard *et al.,* 1999]. It is well known that selenium is an antagonist that moderates the toxic effects of many heavy metals such as arsenic, cadmium, mercury, and lead in organisms [Bierregaard *et al* ., 1999]. Scientists first documented this phenomenon in 1967 [Parizek *et al.,* 1967].

It is increasingly clear that measuring the amount of mercury in the environment or food without considering the protective effects of selenium may provide an inadequate reflection of the potential for health risk [Raymond and Ralston, 2004]. This is important since a higher selenium-to-mercury ratio in fish indicates a greater likelihood that tiny traces of mercury will have no measurable impact on human health [Raymond and Ralston, 2004].

However, a lower selenium-to-mercury ratio in fish indicates a greater likelihood that traces of mercury will have a measurable impact on human health.

1.2 Accumulation of selenium and mercury in marine fish

When polluted freshwater mixes with saline coastal water, chemical processes typically lead to precipitation of many toxic pollutants, including mercury compounds. Estuarine water bodies are thus major repositories for river-borne or watershed derived mercury [Sciencedaily, 2011]. Microorganisms in sediments or water can convert mercury to methyl mercury, a substance that can be absorbed quickly by most organisms. Fish are organisms that absorb great amounts of methyl mercury from surface waters. Consequently, methyl mercury can accumulate in fish and in the food chain that they are part of [Cocoros *et al.,* 1973]. Species of fish that are high on the food chain such as swordfish, king mackerel and tuna contain higher concentrations of mercury than others. As inorganic mercury and methyl mercury are fat soluble, they primarily accumulate in the viscera, although they are also found throughout the muscle tissue [Cocoros *et al.,* 1973]. When this fish is consumed by a predator, the mercury level is accumulated. Since fish are less efficient at depurating than accumulating methyl mercury, fish-tissue concentrations increase over time [Cocoros *et al.,* 1973].

Selenium occurs naturally in the environment. It is released through both natural processes and human activities. Well fertilized agricultural soil generally has about 400 mg/ton of Se since the element is naturally present in phosphate fertilizers and is often added as a trace nutrient [Lenntech, 2011].

Low levels of selenium can end up in sediments or water through weathering of rocks. The water-soluble selenium is then taken up by plants. Consequently, a fish that eats selenium-containing sediments and plants accumulates significant amounts of selenium [Lenntech, 2011].

1.3 The toxic nature of mercury

Mercury exists in two different forms: organic and inorganic mercury. Organic mercury is more toxic than inorganic mercury. Examples of organic mercury are methylmercury, ethylmercury etc. Methylmercury is the most toxic mercury species. Due to toxic effects of methylmercury, the International Program of Chemical Safety has declared methylmercury as one of the most dangerous chemicals in the environment [Gilbert *et al.*, 1995]. Methylmercury affects the central nervous system, kidneys and in severe cases irreversibly damages areas of the brain and ultimately death [Sciencedaily, 2011]. Mercury and its compounds are particularly toxic to infants and pregnant women. Pregnant women exposed to mercury have sometimes given birth to children with serious birth defects [Sciencedaily, 2011].

1.4 Mitigation of mercury toxicity by selenium

Though high selenium or mercury exposures can each individually induce toxicity, co-occurrence of moderately high concentrations of these elements does not produce additive effects but rather, antagonistic effects [Parizek *et al.*, 1967]. That is, their co-occurrence can mutually reduce the toxic effect of each element.

Several molecular forms of selenium have been employed to show that supplemental selenium counteracts mercury toxicity [Ganther *et al.,* 1972; Chen *et al.,* 1973]. It has been shown that mercury toxicity most likely results from mercury inhibiting the activity of selenium-dependent enzymes that normally prevent oxidative damage [Seppanen *et al.,* 2004]. That is intracellular mercury sequesters selenium, forming organic (most likely methylmercury cysteine [$CH_3Hg(CYS)$]) or inorganic HgSe complexes in the body tissues, thereby tying up both elements as highly insoluble compounds [Seppanen *et al.,* 2004; Ralston *et al.,* 2006 ;Peterson *et al.,* 2009].

1.5 The Selenium Health Benefit Value (SeHBV)

Researchers have proposed a new measure of seafood safety called the Selenium Health Benefit Value (SeHBV) that takes the protective role of selenium into account [Kaneko and Ralston, 2007]. Fish with a positive (above zero) SeHBV ratio would be safe to eat, whereas fish with a negative ratio would be unsafe [Kaneko and Ralston, 2007]. Using the proposed criteria, most varieties of ocean fish have positive SeHBV ratios and are thus safe to eat [Kaneko and Ralston, 2007]. Foods that supply more selenium than mercury protect against mercury toxicity and provide selenium-dependent health benefits, whereas those that contain more mercury than selenium are associated with mercury health risks [Kaneko and Ralston, 2007].

1.6 Consumption of canned fish products in Ghana

Canned fish products are highly consumed in Ghana. Restaurants, kenkey sellers, fried rice and other food vendors use canned fish products to prepare their food. Most families also use canned fish products to prepare their food domestically.

Generally, most Ghanaians prefer canned fish products because they are considered to be more hygienic than the other fish products. The fresh fish and other fish products that are sold on the market may be exposed to flies and other contaminants [Bierregaard *et al.*, 1999; The Flipside of mercury, 2006].

Again, Ghanaians like canned fish products because they are already processed and may not need further preparation before consumption. However, there have been numerous reports on mercury accumulation in canned fish in Ghana yet there is no data on selenium and mercury content in these products [Oppong *et al.*, 2010]. Most environmental advocates always talk about mercury levels in fish without talking about selenium content [Raymond and Ralston, 2004]. Measuring the amount of mercury in the food without considering the protective effects of selenium may provide an inadequate reflection of the potential for health risk. There is the need therefore to consider both Hg and Se content in canned fish products since they are always consumed together.

1.7 Problem statement

Interaction between Se and Hg and their molar ratios in fish are essential factors in evaluating health risk associated with dietary Hg exposure due to fish consumption. However, most studies have reported only Hg in fish and its health risk without considering the Se content in the fish. The use of Hg content alone to estimate health risk is inadequate because Se is an antagonist that moderates the toxic effects of Hg in organisms. Canned fish products are widely and highly consumed by Ghanaians. Human populations are exposed to Hg through fish consumption. Yet there is no data on the molar ratios of Se and Hg in canned fish products on the Ghanaian market. This has made it difficult to assess the extent of Se-Hg antagonistic effect, the Hg health risk and the SeHBV associated with the consumption of canned fish products on the Ghanaian market.

1.8 Objectives of the study

1.8.1 Main objective

The study endeavors to provide reliable data on the Se:Hg and Hg:Se molar ratios in canned fish products available on the Ghanaian market and to ascertain the Se health benefit and Hg health risk associated with the consumption of canned fish products by Ghanaians.

1.8.2 Specific objectives

- To determine the concentrations of Se and Hg in thirteen selected canned fish products on the Ghanaian market, using hydride generation atomic absorption spectrometry (HG-AAS) and cold vapour atomic absorption spectrometry (CV-AAS) respectively.
- To evaluate the molar concentrations of Se and Hg in the selected fish products.
- To evaluate the Hg**:**Se and Se**:**Hg molar ratios.
- To assess the Se health benefit and the Hg health risk associated with the consumption of the canned fish products.

CHAPTER TWO

2.0 LITERATURE REVIEW

This chapter discusses the most relevant works done in the field from available literature in comprehensive manner to give clear picture of the current state of research.

2.1 Sources of Mercury

Mercury is a naturally occurring element that is found in air, water and soil [Mathews, 1997]. Mercury is generated naturally in the environment from the degassing of the earth's crust, volcanic emissions and evaporation from water. It exists in several forms: elemental or metallic mercury, inorganic mercury compounds, and organic mercury compounds [Mathews, 1997].

The only ore of mercury is cinnabar (HgS). Both the metal and the sulphide are volatile and traces of the metal are found almost everywhere. Atmospheric mercury is dispersed across the globe by winds and returns to the earth in rainfall, accumulating in aquatic food chains and fish in lakes and rivers [Mathews, 1997]. Mercury occurs in almost all environments as a result of atmospheric transport and deposition and releases from both anthropogenic and natural sources [Fitzgerald *et al.*, 2007; Ullrich *et al.*, 2001; Hong *et al.*, 2012].

In aquatic environments, Hg can be transformed to monomethylmercury (CH_3Hg^+) mainly by biotic processes as abiotic methylation is not common, which can readily enter foodwebs via bioconcentration at low trophic levels and then be subsequently

biomagnified in the food chain via bioaccumulation [Fitzgerald *et al.*, 2007; Ullrich *et al.*, 2001; Hong *et al.*, 2012]. Elevated concentrations of Hg, primarily as CH_3Hg^+, are often reported in fish [Sunderland, 2007; Chen *et al.*, 2008], raising a significant health concern for humans [UNEP, 2002]. Although aquatic predators are primarily exposed to CH_3Hg^+ through the fish diet, inorganic mercury (Hg^{+2}) has been identified in organ tissue, e.g., liver and kidney [Das *et al.*, 2003].

Mercury is also found in many rocks including coal. When coal is burned, mercury is released into the environment. Coal-burning power plants are the largest human-caused source of mercury emissions into the air in the United States, accounting for over 40 percent of all domestic human-caused mercury emissions. EPA has estimated that about one quarter of U.S. emissions from coal-burning power plants is deposited within the contiguous U.S. and the remainder enters the global cycle [USEPA, 2001]. Burning hazardous wastes, producing chlorine, breaking mercury products, and spilling mercury, as well as the improper treatment and disposal of products or wastes containing mercury, can also release it into the environment [USEPA, 2001].

2.2 Sources of Selenium

Se is extensively associated with sulphur in nature. Most elemental selenium comes from the refining of copper sulfides as selenium is a common trace element in these minerals. Selenium is released as SeO_2 and H_2Se into the environment by volcanism. There is no real ore of selenium as these minerals, including native selenium, are far too rare [Amethyst Galleries, 2011]. Ocean fish are among the richest sources of nutritional selenium [Kaneko and Ralston, 2007]. Seafood constitutes 17 of the top 25 sources of

dietary selenium [Kaneko and Ralston, 2007]. Other good sources are foods made from grains grown in selenium-rich soils, such as breads and pasta, and beef grazed on selenium- rich grasses [Kaneko and Ralston, 2007].

2.3 Selenium as a Nutrient

It is now known that selenium is essential for the normal function of many of the systems of the body and selenium deficiency can have adverse consequences on these systems [Behne *et al.,* 2000]. Selenium has antioxidant and anticancer properties and also supports normal thyroid hormone homeostasis, immunity, and fertility [Behne *et al.,* 2000]. Two of the 22 primary amino acids are distinguished by their possession of selenium: selenomethionine and selenocysteine. Selenium is required for the activity of enzymes that are normally present in all cells of all vertebrate forms of animal life [Behne *et al.,* 2000]. Selenium is required for synthesis of selenium-dependent proteins such as selenoproteins and enzymes (selenoenzymes) that are essential for normal metabolic processes, especially in the brain and related tissues [Chen *et al.,* 2003; Schweizer *et al.,* 2004]. Selenoenzymes regulate intracellular redox status, protect against and reverse oxidative damage in brain and related tissues [Kohrle, 1999].

2.4 The Role of Selenium in Our Body

The major role of selenium in our body is to boost the immune system and activation of anti oxidant enzymes [Madhumita, 2012]. These activities are vital for metabolism and body functions. It is concentrated in the kidneys, liver, muscles and the thyroid. Selenium is also known to fight against cancer cells [Madhumita, 2012]. Natural killer cells are formed in the body with selenium intake which destroys any foreign bacteria entering our body [Madhumita, 2012].

2.5 Health Benefits of Selenium

The major health benefits of selenium include prevention of hardening of arteries. Selenium is known to aid pregnant women in healthy fetus development; it also helps to keep the skin looking young [Kaneko and Ralston, 2007]. The pancreas and its function are greatly benefited by the consumption of selenium. Semen production is significantly increased and can be attributed as a health benefit of selenium [Kaneko and Ralston, 2007].

It also reduces the severity of cold, sores and shingles, fights viral infections and helps relieve lupus symptoms **[**Madhumita, 2012**]**. Thyroid hormone is vital for the functioning of every cell in the body, selenium aids in converting this hormone from a less active form thyroxine (T4) to its active form triiodothyronine (T3) [Madhumita, 2012**]**. Selenium's role in combating cancer has garnered a lot of attention recently.

A five-year study conducted at the University of Arizona and the Cornell University showed that consumption of 200 µg (microgram) of selenium daily resulted in 63% fewer prostate tumors, 58% fewer colorectal cancers, 46% fewer lung malignancies, and a 39% overall decrease in cancer deaths [Madhumita, 2012**]**. The health benefits of selenium supplements include making of antioxidants which fight against the free radicals that damage cells resulting in aging. Selenium benefits for hair have shown prevention of dandruff [Madhumita, 2012]. Selenium helps to control the fungus (Malassezia) present on the scalp, which sheds dry skin fragments. Some amount of selenium is also used in the anti-dandruff shampoos. Selenium benefits for skin include prevention of acne and skin cancer [Madhumita, 2012].

2.6 Exposure to Mercury

Mercury is one of the heavy metals of increasing concern as a global pollutant [Raymond and Ralston, 2004]. Human exposure to methylmercury is mainly through fish consumption **[**Raymond and Ralston, 2004**]**. Although adults can experience neurological effects when exposed to high concentrations of methylmercury, advisories have mainly arisen because of the increasing concerns regarding methylmercury's effects on the developing nervous systems of unborn and growing children **[**Raymond and Ralston, 2004**]**. Interestingly, while the placental barrier can stop many toxic elements, methylmercury is an exception, it crosses the placenta and also accumulates at higher concentrations on the fetal side than on the maternal [Iyengar *et al.,* 2001].

Worsening the situation for the developing fetus, mercury also crosses the blood-brain barrier and exhibits long-term retention once it gets across [Kerper *et al.,* 1992].

2.7 The Molar Ratio

A mole is a unit that helps scientists (chemists) to count huge numbers of atoms in a sample of any chemical substance, including mercury and selenium [Mathews, 1997]. Since different chemical atoms weigh different amounts, just comparing the weight of two chemical samples doesn't give an accurate picture of how many atoms are there in each. At the microscopic scale on which chemical reactions take place, all chemicals (including mercury and selenium) react with each other one atom at a time. Therefore, establishing their "molar ratio" thus, comparing them mole-for-mole is a reliable way to determine how many atoms of each are available for these reactions.

Hence, determining the molar ratio of selenium and mercury in fish will help to know whether there is excess selenium to protect against mercury toxicity or there is excess mercury to pose a health risk.

2.8 Selenium and Mercury Interaction

The chemical forms of mercury and selenium are important in the toxicology of both elements. Likewise, the interactions between mercury and selenium also rely, to a large extent, on the chemical state in which the elements exist [Ralston *et al.,* 2006]. It has been shown that Hg toxicity most likely results from Hg inhibiting the activity of Se-dependent enzymes that normally prevent oxidative damage [Seppanen *et al.,* 2004].

This is termed the "selenium sequestration mechanism of mercury toxicity". This is wherein Hg-dependent inhibition of selenoenzymes appears to be the proximate cause of the oxidative damage that occurs as a result of Hg intoxication [Ralston *et al.,* 2006]. That is, intracellular Hg sequesters Se, forming organic, most likely methylmercurycysteine; (MeHg[Cys]) or inorganic HgSe complexes in the brain and other body tissues [Peterson *et al.,* 2009]. This causes the tying up of both elements as highly insoluble compounds. While alternative theoretical explanations for the Se-protective mechanism have been proposed, the selenium sequestration mechanism described above is the most complete [Peterson *et al.,* 2009]. Convincing evidence in support of this mechanism was provided by Seppanen and his group [Seppanen *et al.,* 2004].

Additional support for the Se sequestration mechanism of mercury toxicity is the black, granular material identified as HgSe that accumulates in the brains and livers of higher organisms such as cormorants, sea lions, seals, and whales [Nigro and Leonzio, 1996; Arai *et al.,* 2004; Huggins *et al.,* 2009]. Similar evidence for Se-dependent protections to organisms are derived from the reverse application when otherwise toxic concentrations of Se are counteracted by feeding increased amounts of dietary MeHg [Ganther and Sunde, 1974]. The earliest evidence for this was based on adding mercuric chloride to the diets of chicks containing otherwise toxic concentrations of selenium dioxide [Hill, 1974]. As mercury chloride was increased in the diet, the effects of Se toxicity gradually decreased until a Se:Hg molar ratio of 1:1 was reached. More recent evidence for mutual detoxification responses in crickets, mice and rats have been observed [Watanabe *et al.,* 1999; Raymond and Ralston 2004; Ralston *et al.,* 2006, 2007,

2008]. It is important to note that sulphur and selenium have very similar chemical characteristics but the sulphur (S) in HgS (binding affinity of 10^{39} M) can be displaced in the chemical replacement series by Se to form HgSe (binding affinity of 10^{45} M), which has an even lower solubility product (Ksp = 10^{-59}) than HgS. Björnberg *et al.* (1988) postulated that in areas where S^{2-}or Se^{2-} is abundant in soil and rocks, Hg concentrations in fish would remain low [Yang *et al.*, 2011]. Because selenide has an extremely high affinity constant with Hg^{2+} than sulphide, the combined effect of thermodynamic driving forces and biochemical distinctions between sulphur and selenium metabolism results in the preferential formation of HgSe [Dyrssen and Wedborg 1991]. So long as intracellular molar concentrations of Hg remain sufficiently low in comparison to Se in the tissues selenoenzyme activities in these tissues remain active.

However, as increasing intracellular Hg exceeds Se, portions of the cellular Se essential for selenoenzyme production become sequestered and symptoms of Hg toxicity develop [Hirota, 1986; Raymond and Ralston, 2004]. Therefore, it appears that it is not the concentration of Hg or MeHg present in an organism that is critical, but rather the moles of Hg relative to the moles of Se in the tissues [Hirota, 1986; Raymond and Ralston, 2004].

Likewise, it appears that Se:Hg molar ratios that are less than 1:1 increase Hg toxicity potentials, while Se:Hg molar ratios that approach or exceed 1:1 increasingly protect against Hg toxicity [Kasuya, 1976]. Provided cellular selenoenzyme activities are not diminished or interrupted by Hg toxicity, their protection against oxidative damage in vulnerable tissues such as brain is reduced or prevented [Kaneko and Ralston, 2007].

From this perspective, mass action effects explain the benefits of providing supplemental dietary Se in overcoming MeHg-dependent inhibition of selenoenzymes [Kaneko and Ralston, 2007]. We assume this protective mechanism is fully functional in fish. Since certain marine species contain Hg concentrations that would be expected to produce toxic effects, yet while they could harbor some neurological effects not readily observable, they show no outward sign of Hg toxicity [Kaneko and Ralston, 2007]. In addition to high Hg concentrations, these fish have a molar concentration of Se greater than that of Hg (Se:Hg >1).

2.9 Selenium-Mercury Interaction and possible Mechanisms of Protection

The actual mechanisms of interaction between mercury and selenium have not been well established [Cuvin-Aralar *et al.*, 1990]. Available information on these mechanisms are inferences from observed results of a number of different studies. A few examples of the protective effect of Se against Hg are: selenium- mercury complex formation, prevention of oxidative damage, redistribution of mercury in the presence of selenium, conversion of toxic forms of mercury to other forms, and competition for binding sites between mercury and selenium [Frisk *et al*,. 2001]. The details of these possible mechanisms are discussed further in the next sections.

2.9.1 Selenium-Mercury Complex Formation

Mercuric chloride and selenite administered at the same time to rats significantly altered plasma protein binding of selenium and mercury compared with those which were given each element alone [Cuvin-Aralar *et al.,* 1990]. After simultaneous administration, both mercury and selenium were present in the plasma in much greater quantities due to their binding to a single plasma protein [Burk *et al.,* 1974]. Irrespective of variations in mercury and selenium dose, the molar ratio of selenium to mercury in the protein remained close to unity.

Further analysis showed that selenium was attached to sulfhydryl groups and that mercury was attached to the selenium. This mercury-selenium-protein complex is presumed to play a role in preventing acute inorganic mercury toxicity by binding the mercury and thus, preventing it from reaching target tissues [Burk *et al.,* 1974; Frisk *et al,* .2001]. This principle might also explain the consistent 1:1 molar ratio between mercury and selenium found in tissues of organisms [Koeman *et al.,* 1975]. Studies with rabbit blood showed that methylmercury bound to the proteins of rabbit blood was converted *in vitro* to free methylmercury soluble in benzene by the addition of selenite under physiological conditions [Sumino *et al.,* 1977].

Further studies have shown that when methyl mercuric chloride and sodium selenite are added to rabbit blood, benzene extraction shows a 2:1 molar ratio of mercury- to-selenium. Later studies show that both mercury and selenium form a single compound identified as bis (methyl mercuric) selenide, ($[CH_3Hg]_2Se$) [Naganuma and Imura, 1980; Frisk *et al,.* 2001]. The formation of this compound depends on the conversion of selenite to selenide [Magos *et al.,* 1979].

The participation of glutathione in the formation of bis (methyl mercuric) selenide was also investigated [Naganuma and Imura, 1980]. Glutathione is assumed to reduce sodium selenite chemically.

Results of addition of glutathione to methyl mercuric chloride and sodium selenite in blood suggest that glutathione mediates the production of bis (methyl mercuric selenide) in the blood [Cuvin-Aralar *et al.,* 1990]. The exact mechanism by which glutathione mediates the formation of this reaction product is still been investigated [Cuvin-Aralar *et al.,* 1990]. It is assumed, however, that this plays a role in the protective effect of selenium against methyl mercuric toxicity. It appears that the processes involved in the formation of a mercury-selenium-protein complex and bis methyl mercuric selenide in the blood are quite different [Cuvin-Aralar *et al.,* 199]. This is so because the formation of the two complexes results in different molar ratios between mercury and selenium [Cuvin-Aralar *et al.,* 1990].

Again, there are two different forms of mercury involved in the formation of these complexes, although there is a possibility that methyl mercuric chloride can also form mercury-selenium-protein complex [Hirota *et al.,* 1980]. However, it is certain that inorganic mercury has to undergo methylation before it can form bis methyl mercuric selenide. It is not clear at this point whether the processes for the formation of these two complexes occur simultaneously or are mutually exclusive [Cuvin-Aralar *et al.,* 1990].

2.9.2 Prevention of Oxidative Damage

Selenium is a component of glutathione peroxidase which is an antioxidative enzyme. Mercury is known to have an inhibitory effect on the activity of this enzyme [Hirota *et al.*, 1980]. This explains part of the damaging effect of mercury, particularly in liver and nervous tissue.

The possible role of the free radicals formed from the homolytic breakdown of methylmercury in inducing neurotoxic effects has been proposed [Ganther, 1978]. Methylmercury would be taken up by membranes in target tissues, such as the brain, in close proximity to lipids and then initiate a chain reaction peroxidation of various lipid constituents as a result of methylmercury's tendency to undergo homolytic fission [Ganther, 1978]. Without selenium treatment, methylmercury will thus inhibit glutathione peroxidase activity, making it unable to decompose peroxides that may initiate methylmercury breakdown into methyl and mercury free radicals and consequently this will result in tissue damage [Cuvin-Aralar *et al.*, 1990].

Studies have shown that treatment with selenium will totally alleviate the inhibitory effect of methylmercury on glutathione peroxidase, by securing the integrity of the biological components of cells and tissues through antioxidation [Chang and Suber, 1982]. Other studies have reported that they observed no evidence of breakage of the C-Hg bond in a number of tissues [Sheline and Schmidt-Nielsen, 1977], but this does not necessarily negate Ganther's free radicals hypothesis. Even if there was homolytic fission of methyl mercury into CH_3 and Hg free radicals, such radicals do not have time to redistribute independently to other tissues.

Because of their highly unstable nature, they would immediately interact with other molecules, for instance, with lipids and other tissue components, and eventually become bound to them [Ganther, 1978].

2.9.3 Redistribution of mercury

Mercury uptake is not diminished by the presence of selenium [Stillings *et al.,* 1974]. In fact, some studies indicate that in certain instances, mercury uptake is enhanced in the presence of selenium [Stillings *et al.,* 1974]. It was also shown that selenium does not enhance mercury elimination. A number of observations to the contrary have been presented. Enhancement of mercury retention by selenium has been observed [Stillings *et al.,* 1974]. These findings indicate that the mechanisms for the observed protective action of selenium against mercury toxicity lie along different lines [Stillings *et al.,* 1974]. It is believed that the rechanneling of mercury from one organ to another and from one sub-cellular fraction to another is one of the general mechanisms involved in the protective action of selenium against mercury toxicity. This was strengthened by observations that toxic levels of mercury and selenium were found in animals not showing signs of mercury or selenium poisoning [Wagemann and Muir, 1984]. Earlier studies showed that selenium promotes the redistribution of mercury from highly sensitive organs and tissues (like the kidney) to less sensitive ones (like the muscles) [Chen *et al.,* 1974; Sheline and Schmidt-Nielsen, 1977].

Reduction in mercury levels in the kidney may explain Parizek and Ostadalova's results, wherein they found neither macroscopic nor histological damage to the kidney of rats treated with sublet all levels of mercury and selenium [Parizek and Ostadalova, 1967].

In the sub-cellular soluble fraction, mercury is bound chiefly to metallothionein, a low molecular weight protein [Winge *et al.,* 1975]. The formation of metallothionein is induced by the presence of certain metals, including mercury [Winge *et al.,* 1975]. Aside from decreases in mercury levels in the soluble fraction, the presence of selenium also resulted in the diversion of the remaining mercury from metallothionein to high molecular weight proteins [Chen *et al.,* 1974; Komsta-Szumska and Chmielnicka, 1977].

This suggests that selenium, in one way or another, blocks the binding of mercury to metallothionein or it may even inhibit the induction of metallothionein by mercury bound to proteins. It was also demonstrated that selenium is effective in releasing mercury bound to cysteine [Sumino *et al.,* 1977]. Since cysteine is a major component of the protein metallothionein and mercury is known to interact with the surfhydryl group of this amino acid [Winge *et al.,* 1975], the blocking of the induction of metallothionein by selenium would thus leave mercury free to bind with other proteins, possibly to those with surfhydryl groups. The higher molecular weight proteins to which mercury is diverted are not yet characterized but they are presumed to be less sensitive to mercury.

In contrast to the preceding studies, [Burk *et al.,* 1977] it was found that the presence of dietary levels of selenium facilitated the accumulation of mercury in the kidney. Moreover, no change in the mercury-binding pattern was reported.

This study led to the assumption that selenium may mediate the binding of mercury to metalothionein or may even be a permissive factor in the induction of metalothionein by mercury. It is interesting to note that this is not in agreement with other studies on the effects of selenium on mercury redistribution.

The redistribution of mercury from more sensitive targets to less sensitive sites cannot fully explain the results of a number of other studies [Burk *et al.,* 1977]. For instance, the brain is also highly sensitive to mercury and the presence of selenium enhances mercury accumulation in this organ [Burk *et al.,* 1977]. It is apparent that redistribution of mercury cannot satisfactorily explain the reduction of neurological damage induced by selenium treatment and that more complex mechanisms are involved in the interaction between these two elements [Cuvin-Aralar *et al.,* 1990].

2.9.4 Conversion of Toxic Form of Mercury

Different forms of mercury have different toxicities. Methylmercury is known to be more toxic than most other forms. The conversion of methylmercury to less toxic forms may be one of the possible mechanisms of detoxification. It has been established that a small amount of methlymercury can be converted to inorganic mercury [Norseth and Clarkson, 1970]. Inorganic mercury is less toxic than methylmercury and has a shorter biological half-life due to its preferential excretion in the faeces [Norseth and Clarkson, 1971]. It has been suggested that the protective effect of selenium and cysteine against methylmercury may be due to an increased rate of conversion of methylmercury to inorganic mercury [Stillings *et al.,* 1974].

Results indicating that this does not occur have also been reported for the killifish [Sheline and Schmidt-Niesen, 1977]. Sheline and Schmidt-Niesen (1977) tested for indications of whether demethylation and conversion to inorganic mercury occur by determining whether a breakage of the carbon-mercury bond of methylmercury occurs.

Carbon-14 and Mercury-203 were used to label methylmercury and determined the tissue distribution of the two isotopes. Their results showed that there was no difference in the distribution of the two isotopes in the tissues. Hence, the conclusion that no breakage of the carbon-mercury bond of methylmercury had occurred [Sheline and Schmidt-Niesen, 1977]. Studies on the effect of dietary selenite on the activity of carbon-mercury cleavage enzymes in rat liver and kidney showed that the activity of the methylmercuric chloride cleavage enzyme was unchanged [Fang, 1974]. Again, there was also no evidence that methylmercury is converted to dimethylmercury or to inorganic mercury [Sumino et al., 1977].

2.9.5 Competition for Binding Sites

The variability of mercury-to-selenium ratios in fish compared with the concentrations of these two elements in the environment led to the assumption that mercury and selenium compete for the same receptors located in the animal tissue [Cuvin-Aralar *et al.,* 1990]. This could also explain their toxicological antagonism. It is also believed that these binding sites are selenium receptors which increase in numbers with age [Leonzio *et al.,* 1982]. It is likely that these receptors can be occupied by mercury in proportion to its bioavailability in the environment [Leonzio *et al.,* 1982].

The idea of competition for binding sites has also been used to explain the rates of elimination of these two elements. The slower rate of excretion of both mercury and selenium when present together may be due to the competition between the two elements for the same carrier protein at transport sites [Lucu and Skreblin, 1981].

The fact that both selenium and mercury have high affinities for sulfhydryl groups of amino acids lends credibility to the idea of competition for carrier proteins, as well as other binding sites [Lucu and Skreblin, 1981].

2.10 Evidence of selenium protection against mercury

Selenium was first shown to reduce the toxicity of mercury given to rats in 1967 [Parizek *et al.,* 1967]. The Science Journal published the results of a study in which the selenium in canned tuna protected Japanese quail from mercury toxicity [Ganther *et al.,* 1972]. This study found that samples of tuna with higher mercury content also contained higher selenium levels.

In 1989, Swedish scientists reported that adding selenium to a lake over a period of three years lowered the mercury levels in the native fish by over 75% [Paulsson *et al.,* 1989]. In 1990, the United States Fish and Wildlife Service published the results of its National Contaminant Biomonitoring Program from 1976 to 1984.

The programme measured the concentrations of seven chemicals (including selenium and mercury) in three hundred and fifteen (315) fish caught at one hundred and nine (109) different locations nationwide [Schmitt *et al.,* 1990]. In this broad survey, selenium was 28 times more than mercury and no fish sample had a Se: Hg ratio of < 1.46: 1. In 1991, a review of selenium and mercury interactions provided an explanation for how surplus of selenium could protect against mercury, suggesting that the two chemical elements may "compete for the same receptors located in animal tissue" [Cuvin-Aralar *et al.,* 1990].

A Danish team, in 1999, described laboratory experiments in which lake trout were given mercury injections along with dietary selenium. Compared to other fish that received only the mercury, the selenium-injected fish excreted much more of the mercury from their bodies [Bierregaard *et al.,* 1999; The Flipside of mercury, 2006].

In the year (2000) a group of Greenland scientists published the results of mercury and selenium tests performed on the muscles and organs of healthy fish, shellfish, birds, seals, whales, and polar bears. They found that "selenium was present in a substantial surplus compared to mercury in all animal groups and tissues" [Dietz *et al.,* 2000]. In 2001, forty-five (45) researchers at Laurentian University in Ontario reported that selenium deposits (from metal smelters) into lake water greatly reduced the absorption of mercury by microorganisms, insects, and small fish [Chen *et al.,* 2001; The Flipside of mercury, 2006].

Also in 2006, two McGill University researchers documented how feeding selenium to rats lowered the impact of dietary mercury. "Antioxidant nutrients" like selenium, they wrote, "may alter methylmercury's reproductive and developmental toxicity" [Beyrouty et al., 2006 ; The Flipside of mercury, 2006].

2.10 Atomic Absorption Spectrometer (AAS)

An Atomic Absorption Spectrometer is an instrument which is used to analyze the concentrations of metals in solution [Varian Australia, 1997].

Many elements can be determined directly over a wide range of concentrations from ppb to percent levels, with good precision. Sample preparation is generally simple and frequently involves little more than dissolution in an appropriate acid [Varian Australia, 1997].

2.10.1 Principles of Atomic Absorption Spectroscopy [Varian Australia, 1997 with Cresser.et al, 1993]

The following are the basic principles of Atomic Absorption Spectroscopy:

- All atoms can absorb light.

- The wavelength at which light is absorbed is specific for each element.

- If a sample containing selenium, for example, together with elements such as lead and copper is exposed to light at the characteristic wavelength for selenium, then only the selenium atoms will absorb this light.

- The amount of light absorbed at this wavelength will increase as the number of atoms of the selected element in the light path increases, and is proportional to the concentration of absorbing atoms.

- The relationship between the amount of light absorbed and the concentration of the analyte present in known standards can be used to determine unknown concentrations by measuring the amount of light they absorb [Varian Australia, 1997 and Cresser *et al.,* 1993].

2.10.2 The Absorbance - Concentration Relationship

When the absorbance is measured, this value can then be related to the concentration of an element in solution [Varian Australia, 1997 and Cresser *et al.*, 1993]. The relation between light absorption and analyte concentration is called the Beer-Lambert law.

2.10.2.1 Beer-Lambert Law

States that:

- The portion of light absorbed by a transparent medium is independent of the intensity of the incident light, and each successive unit thickness of the medium absorbs an equal fraction of the light passing through it [Cresser *et al.*, 1993].
- The light absorption is proportional to the number of absorbing species in the sample [Cresser *et al.*, 1993].
- Effectively for AAS, this means that the amount of energy (light) absorbed is proportional to the concentration of atoms in the atomizer. Thus if a concentration of atoms 'c' produced an absorbance 'a', a concentration '2c' would produce an absorbance '2a' [Varian Australia, 1997 and Cresser *et al.*, 1993].

The combined Beer-Lambert law can be expressed as:

$\log_{10}(\frac{I_o}{I_t})$= a * b * c ---------- (2.1)

Where:

I o = incident light intensity

I t = transmitted light intensity

a = absorption coefficient (absorptivity)

b = length of absorption path

c = concentration of absorbing atoms

For a given set of conditions, a and b are constants. The path length, b, will change if different burners are used, as an air/acetylene burner has a path length of 100 mm compared to 60 mm for the nitrous-oxide/ acetylene burner. If this expression is plotted, and a curve of absorbance versus concentration is drawn, Beer's Law predicts that a straight line will result [Varian Australia, 1997 and Cresser *et al.*, 1993].

2.10.3 Hydride Generation

Hydride generation is a technique in which the analyte is reacted with a reductant, usually sodium borohydride, to form a volatile hydride of the analyte. This is reduced to free atoms in a quartz cell mounted in the optical path using heat from a flame or an electrical heater [Smith *et al.*, 1983].

2.10.4 Vapour Generation

In recent years, it has become more important to be able to determine elements such as arsenic, selenium, antimony and mercury at low levels in the environment [Smith *et al.*, 1983].

Vapour generation is an extremely sensitive method for determining mercury and certain hydride-forming elements which form stable metal hydrides such as arsenic, selenium, antimony, bismuth, tellurium, and tin [Smith et al., 1983].

These elements may be determined by chemically reducing the element to the gaseous hydride and then dissociating the hydride in a heated quartz tube. This is the principle of operation of vapor generation [Varian Australia, 1997 with Smith *et al.,* 1983]. Vapour generation is often preferred to graphite furnace analysis for arsenic, selenium and mercury because of the improved speed of analysis and the lack of background absorbance signals. Vapor generation AAS detection limits are usually in the sub parts per billion (µg/L) ranges [Bennett *et al.,* 1983]. The improved sensitivity of the vapour generation technique is achieved by virtue of the 100 % sampling efficiency. All of the analyte in the sample solution used in the reaction is chemically reduced and transported to the sample cell for measurement [Tsalev *et al.,* 1990].

This process also effectively separates the analyte element from its chemical matrix, eliminating matrix interference effects in the atomization process and minimizing background absorption [Bennett *et al.,* 1983]. A number of different vapour generation systems are commercially available. Varian's Vapour Generation Accessory employs a peristaltic pump to provide continuous flow vapor generation. In this technique the sample flow is combined with a flow of concentrated acid and sodium borohydride solution (the reductant), before being pumped into a reaction coil [Bennett *et al.,* 1983].

Volatile hydrides are formed for a range of elements and these hydrides are separated from the flow of solutions using a gas liquid separator. The gaseous hydrides then pass to a heated quartz cell aligned in the optical path [Bennett *et al.,* 1983].

The quartz cell is usually heated by an air/acetylene flame. The hydride is atomized in the cell and breaks down into the analyte and hydrogen. This allows the atomic absorption of the analyte to be measured [Bennett *et al.*, 1983].

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Sampling and sample preparation

Fifty-two samples from thirteen commercially-available and widely consumed canned fish products were purchased from various retail outlets in Accra. For each brand, four (4) samples were purchased. The purchased canned fish products were transported to the laboratory at the Nuclear Chemistry and Environmental Research Centre of the Ghana Atomic Energy Commission (GAEC) for processing and analysis. The brand name, type of fish, and the country of manufacture of the canned fish products used for the study are presented in Table 3.1. A photograph of the canned fish products is also presented at appendix 2.

At the laboratory, all the four samples for each brand of canned fish product were opened and the contents carefully and quantitatively poured into a labelled acid-washed polyethylene containers to form a composite sample. The polyethylene containers were placed in a freezer at -20 ºC. This was followed by freeze-drying of the samples using the Christ Gamma 1-16 lyophilizator (Adotey *et al.,* 2011). After lyophilization, samples were milled and homogenized in a blender with Teflon-coated parts to obtain fine powdery samples (Garcia *et al.,* 2001; Robberecht *et al.,* 2002). Each powdery fish sample was stored in 200 mL polyethylene containers, subsequently the containers were placed in hermetically-closed polyethylene bags and stored in a refrigerator at 4 ^{0}C.

Aliquots of the samples were taken and used for all intended analysis. Canned fish products containing excess fat (vegetable oil) were defatted prior to lyophilization. The

de-fatting process was done according to the method described by Šlejkovec *et al.*, (1999). The defatting procedure is as follows: the fish product carefully and quantitatively transferred into a 250 mL round-bottomed flask fitted to a rotary evaporator. Appropriate quantity of diethyl ether was added to the fish to cover the fish in the flask. The round-bottomed flask was lowered into a water bath temperature at 45 ºC. The fish was defatted by extraction of the fat with diethyl ether. This was followed by evaporation of the defatted fish to dryness. The dried fish was frozen, lyophilized, milled and homogenized in a blender with Teflon-coated parts to obtain fine powdery samples.

Table 3.1: Canned fish products purchased from the retail outlets

Brand name	Code	Type of fish	Country of origin
Empress Mackerel	EM	Mackerel	Thailand
Royal Boat Pilchards	RBP	Pilchard	China
Star Mackerel	SM	Mackerel	China
African Queen Mackerel	AQM	Mackerel	Thailand
Obaapapa Sardines	OS	Sardine	Indonesia
Kaakyire Mackerel	KM	Mackerel	China
Star Kist Tuna	SKT	Tuna	Ghana
Ohene Sardines	OHS	Sardine	Indonesia
Joly Omega-3 Sardines	JOS	Sardine	Unknown
Teacher Tuna Flake	TTF	Tuna	Thailand
Vega Tuna Flake	VTF	Tuna	Thailand
Belma Sardines	BS	Sardine	Morocco
Titus Sardine	TS	Sardine	Morocco

3.2 Determination of Se and Hg by HG-AAS

All glassware were soaked overnight in 10% HCl, rinsed with double distilled water and dried in an oven before been used for analysis (Khansari *et al.*, 2005). Total Se and total Hg were determined using the hydride generation atomic absorption spectrometry (HG-AAS) and cold vapour atomic absorption spectrometry (CV-AAS) respectively. The HG-AAS and CV-AAS systems consisted of an AA 240FS fast sequential atomic absorption spectrometer (with a deuterium background corrector) equipped with a VGA-77 vapour generator (Varian, Australia). Varian's Vapor Generation Accessory (VGA-77) employs a peristaltic pump to provide continuous flow vapor generation. The radiation sources were the hollow cathode lamp of Se (wavelength 196 nm; spectral slit width 1.0 nm; lamp current 10 mA), and the hollow cathode lamp of Hg (wavelength 253.7 nm; spectral slit width 0.5 nm; lamp current 4 mA) (Varian, Australia). The air-acetylene flame atomizer was made up of air as oxidant (flow rate: 13.50 L/min) and acetylene as fuel (flow rate: 2 L/min).

ETHOS 900 microwave digester (Milestone, USA) was used for digestion of fish samples. Calibrated weighing balance Mettler Toledo AE 163 (Zurich, Switzerland) was used for weighing of samples and standards.

All solutions were prepared from analytical grade reagent: Commercially available standard stock solution of selenium [(1000 mg Se L^{-1} in 2% (w/w) HNO_3, CertiPUR®, Merck, Germany], and Commercially available standard stock solution of mercury [(999 ± 4 µg Hg mL^{-1} in 1.4% (w/w) HNO_3, Spectrascan, Teknolab AB, Sweden] were used for the preparation of standard solutions of selenium and mercury respectively.

Sodium borohydride ($NaBH_4$, Fluka, Switzerland) was used to generate the hydride; Nitric acid (65% HNO_3, Merck, Germany);

Hydrochloric acid (37 % HCl, Merck, Germany) and Hydrogen peroxide (30%, H_2O_2, Merck, Germany) were used to digest the fish samples. Calibration standards for Se (0.2, 0.4, 0.6 mg L^{-1}) and Hg (0.1, 0.25, 0.5 mg L^{-1}) were prepared daily by appropriate dilution of the respective standard stock solutions for Se and Hg.

The validity of the HG-AAS and CV-AAS methods for Se and Hg determination was checked by analysis of a National Research Council of Canada (NRCC), certified reference material DORM-2 (Dogfish Muscle). The reference material was analyzed together with the samples under the same experimental conditions.

3.3 Digestion

Digestion of fish samples was done according to the procedure described by Hoenig et al., (1998). The procedure is as follows: 6 mL of HNO_3 (65%), 3 mL of HCl (37%) and 0.25 mL of H_2O_2 (30%) were added to about 500 mg aliquot of lyophilized fish sample in Teflon digestion tubes, the tubes were covered tightly and placed in the ETHOS 900 microwave digester. The fish samples were digested using a four-step digestion procedure as described in (Table 3.2). At the end of the digetion, the digest was cooled, transferred into clean 25 mL volumetric flask. Aliquots of the digest was used for determination of selenium and mercury.

Table 3.2: Microwave digestion programme used for digestion of fish samples

Digestion Step	Digestion Time (min)	Microwave			
		Power (W)	Pressure (bar)	Temperature 1 (^{o}C)	Temperature 2 (^{o}C)
1	5	250	100	400	500
2	1	0	100	400	500
3	10	250	100	400	500
4	5	450	100	400	500

Temperature 1 and temperature 2 represents the initial and final digestion temperatures

3.2.1 Chemical analysis

The scheme for the determination of Se and Hg by HG-AAS and CV-AAS is presented in Fig 3.1.

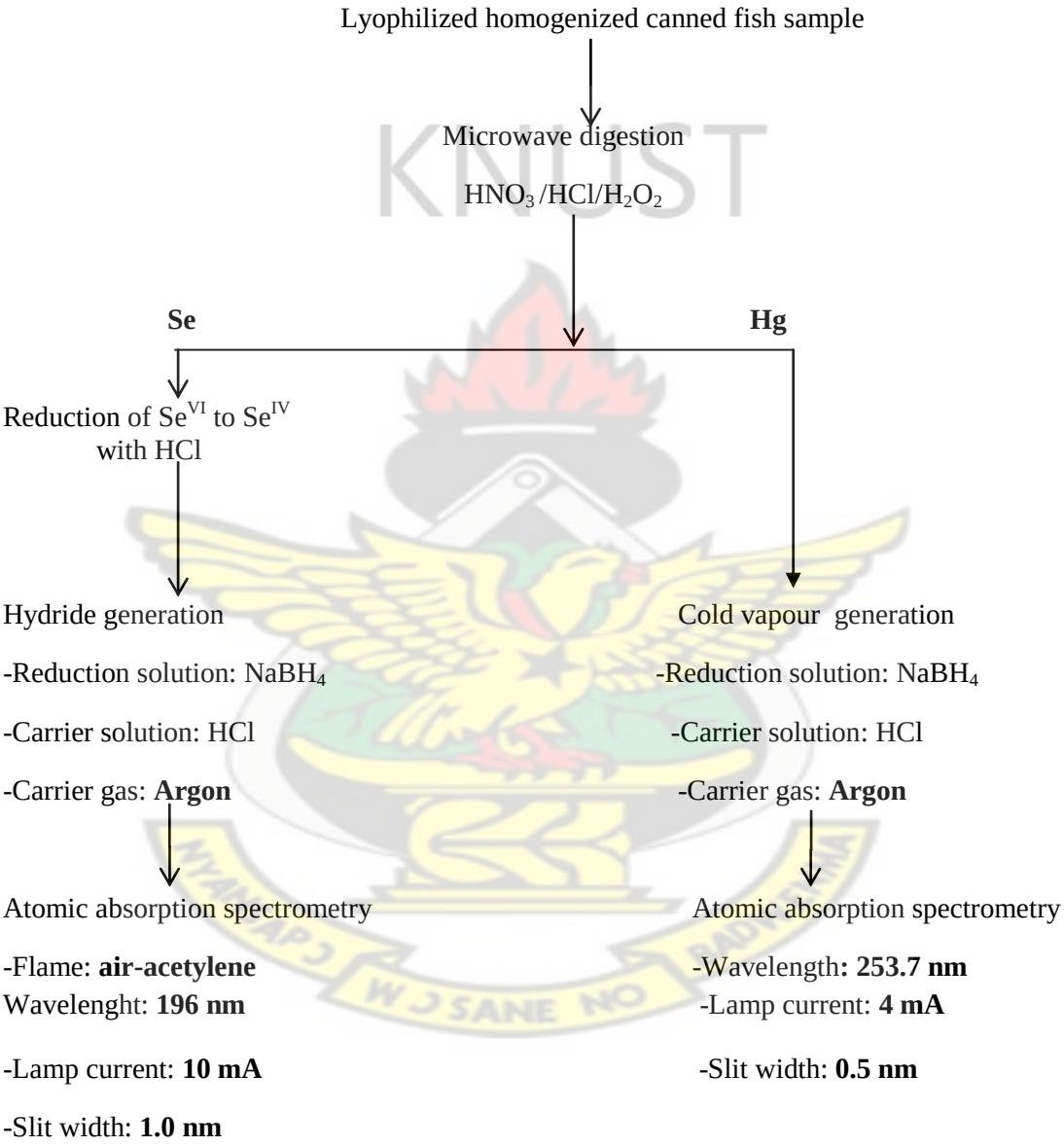

Fig 3.1 Schematic flow diagram for Se and Hg determination by HG-AAS

3.2.2 Determination of Se

Reagents for Se hydride generation

For Se determination, hydride generation was performed with a 0.6% (w/v) of $NaBH_4$ as the reductive solution. This solution was prepared daily or more frequently if required. 6 M HCl was used as the carrier solution.

REDUCTION OF SELENATE (Se^{VI}) TO SELENITE (Se^{IV})

To reduce Se^{VI} to Se^{IV}, 2.5 mL of 6 M HCl was added to the digest and heated at 100 °C for 10 minutes. The reduced solution was cooled to room temperature.

$$SeO_4^{2-} + 2HCl \rightarrow SeO_3^{2-} + H_2O + Cl_2 \quad (3.1)$$

The reduction was necessary because under acidic conditions selenite species are reduced to hydrogen selenide, whereas selenate species are not reduced (Tamari et al., 1992). Therefore before hydride generation is undertaken; all selenium must be reduced to selenite.

3.2.3 Calibration of Se standard for HG-AAS system

The HG-AAS system was calibrated with Se standard calibrants (0.2, 0.4, 0.6 mg Se L^{-1}) and the absorbance obtained were used for linear regression analysis (plot of absorbance against the concentration of the calibrants) [Fig 3.1]. The concentration of Se was deduced from the equation of the regression line.

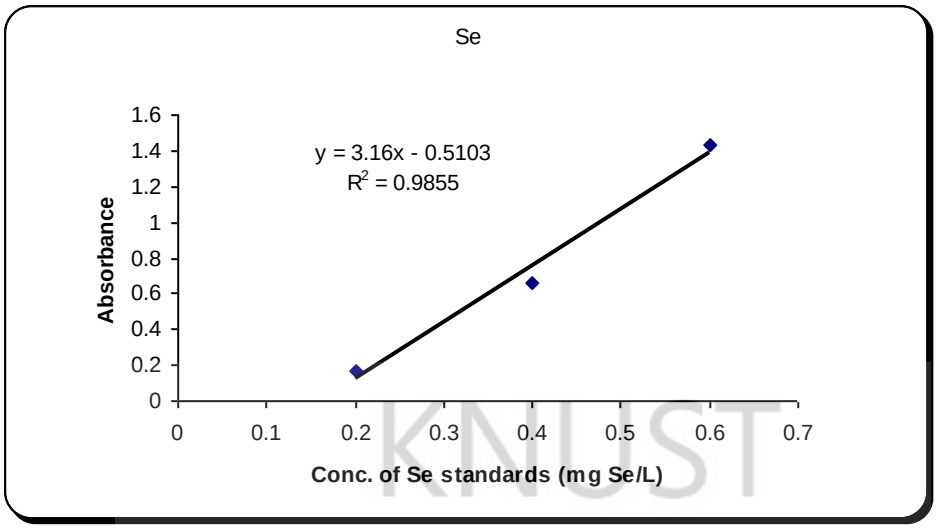

Fig 3.2 Linear regression line for the calibration of the HG-AAS method for Se determination

3.3 Hydride generation and atomic absorption measurement

The continuous flow approach of an HG-AAS system was used to merge sample solution and reagents. The sample solution (flow rate: 5 mL/45 sec) was mixed in a PEEK (polyetheretherketone) cross connector with both HCl (flow rate: 5 mL/45 sec) and $NaBH_4$ (flow rate: 5 mL/45 sec) solutions (both solutions pumped and added with the peristaltic pump) and pumped into the reaction coil. Hydride generation was performed in 2 M HCl solution. The carrier solution (0.05 M HCl) and the sample solution were mixed in a PEEK (polyetheretherketone) cross connector with HCl (2 M) and $NaBH_4$. The hydrogen selenide, H_2Se, and hydrogen which resulted from the mixing in the cross piece were separated from the liquid in a gas-liquid separator, swept from it with argon and dried in a Permapure dryer.

During the mixing of the solutions the following chemical reactions took place:

The tetrahydroborate ion, BH_4^-, converts Se^{IV} into the hydride form (H_2Se).

$$4H_2SeO_3 + 3BH_4^- + 3H^+ \rightarrow 3H_3BO_3 + 3H_2O + 4H_2Se \quad (3.2)$$

The gaseous hydride formed was separated by a gas-liquid separator, then transferred with a flow of argon, and passed through a hygroscopic membrane. The hydride was atomized in hydrogen diffusion flame.

The hydride and excess hydrogen were swept out of the generation vessel by a stream of argon (flow rate 13.5 mL min^{-1}) into a chemically hydrogen diffusion flame into the AAS detection system. In the detection system, H_2Se was atomized in the air-acetylene flame (also fed by the excess hydrogen generated) aligned in the light path of a Se lamp in the atomic absorption spectrometer. Absorbance measurements were recorded and the concentration deduced from the regression line.

3.4 Calculation of concentration

The concentration of Se in each lyophilized fish sample was obtained from the equation of the regression line. The concentration of Se in the sample (mg L^{-1}) was done using the relation (3.4):

$$Conc_{Se} = \frac{Conc_{Calib.} \cdot D_f \cdot V_{Sample}}{M_{Sample}} \quad (3.4)$$

Where: D_f is dilution factor,

$Conc_{calib}$ is the concentration from calibration curve (mg L^{-1}),

$Conc_{Se}$ is the concentration of analyte (Se) (mg g^{-1}), and

V_{sample} is the volume of the sample (L)

3.5 Determination of Hg

Reagents for cold vapour generation of Hg

Cold vapour generation for Hg determination was performed using 0.3% (w/v) of $NaBH_4$ as reductive solution. 5 M HCl was used as carrier solution.

3.6 Calibration of Hg standard for CV-AAS

The CV-AAS system was calibrated with Hg standard calibrants (0.1, 0.25, 0.5 mg Hg L^{-1}) and the absorbances obtained were used for linear regression analysis (plot of absorbance against the concentration of the calibrants) [Fig. 3.2]. The concentration of Hg was deduced from the equation of the regression line.

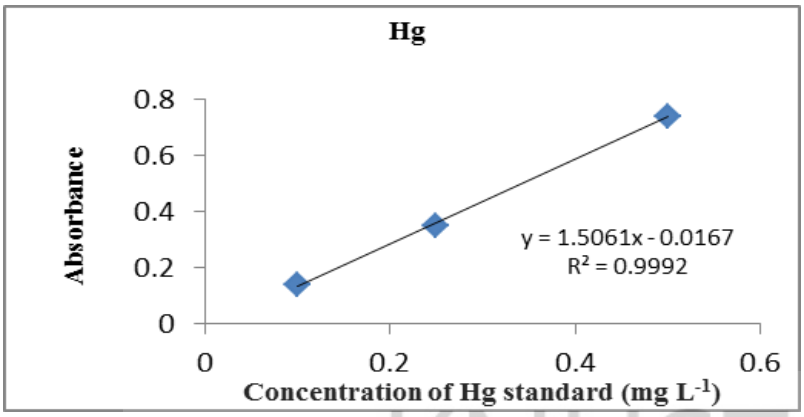

Fig 3.3 Linear regression line for the calibration of the CV-AAS method for Hg determination

3.7 Cold vapour generation and atomic absorption measurement

Continuous flow hydride generation atomic absorption spectrometry was used for the determination of Hg in water samples. The experimental set-up was the same as outlined for the determination of Se. A brief description of the determination of Hg is as follows:

During the mixing, the tetrahydroborate ion, BH_4^- converts Hg into the elemental state (Hg^0). Furthermore, the tetraborohydrate is hydrolyzed in the presence of HCl producing considerable hydrogen:

$$BH_4^- + 4Hg^{2+} + 3H_2O \rightarrow 4Hg^0 + H_3BO_3 + 7H^+ \quad \text{(3.5)}$$

$$BH_4^- + H^+ + 3H_2O \rightarrow H_3BO_3 + 4H_2 \quad \text{(3.6)}$$

The gaseous Hg^0 formed together with the hydrogen gas generated were separated from the liquid in the A-shaped gas-liquid separator component of the vapour generator, and transferred with a flow of argon gas (flow rate: 13.5 mL min^{-1}) .

The liquid goes to waste and the gaseous Hg and excess hydrogen formed were swept out of the vapour generation vessel by the argon gas into the AAS detection system. The Hg vapour produced (Hg^0) was directed into a quartz cell positioned in the optical path for measurement by atomic absorption. Absorbance measurements were recorded and the concentration deduced from the regression line.

Calculations

(a) Calculation of molar concentrations of Se and Hg

The molar concentration of Se was calculated from the relation:

$$Molar\ concentration\,(Se) \quad = \quad \frac{Molar\ concentration\ of\ Se}{Molar\ mass\ of\ Se} \tag{3.7}$$

The molar concentration of Hg was calculated from the relation:

$$Molar\ concentration\,(Hg) \quad = \quad \frac{Molar\ concentration\ of\ Hg}{Molar\ mass\ of\ Hg} \tag{3.8}$$

(b) Calculation of Se:Hg molar ratio

The Se:Hg molar ratio was calculated from the relation:

$$Se : Hg \quad = \quad \frac{Molar\ concentration\ of\ Se}{Molar\ concentration\ of\ Hg} \ \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \tag{3.9}$$

(c) Calculation of Hg:Se molar ratio

The Hg:Se molar ratio was calculated from the relation:

$$Hg:Se = \frac{Molar\ concentration\ of\ Hg}{Molar\ concentration\ of\ Se} \quad \text{..... (3.10)}$$

(d) Calculation of Se health benefit value (SeHBV)

To better describe and integrate selenium-specific nutritional benefits in relation to potential mercury-exposure risks presented by a given type of seafood, the proposed selenium health benefit value (SeHBV) is calculated as follows [Kaneko and Ralston, 2007]:

$$SeHBV = [(Se:Hg)\cdot(Total\ Se) - (Hg:Se)\cdot(Total\ Hg)] \quad \text{.....(3.11)}$$

(e) Calculation of Free Se

The free Se is obtained by simply subtracting the molar concentration of Hg from the molar concentration of Se. The free Se is calculated as follows:

$$Free\ Se = [(Molar\ Concentration\ of\ Se) - (Molar\ Concentration\ Hg)] \quad \text{.....3.12}$$

3.8 Validation of HG-AAS and CV-AAS methods for Se and Hg determination respectively

The validity of the hydride generation atomic absorption spectrometric (HG-AAS) methods for selenium and cold vapour atomic absorption spectrometric (CV-AAS) for mercury determinations respectively were checked by analyzing compositionally

appropriate certified reference material, DORM-2 (Dogfish muscle) issued by the National Research Council of Canada (NRCC). The certified reference material was analyzed together with the samples under the same experimental conditions. The measured Se and Hg contents in DORM-2 were in good agreement with the respective NRCC's certified values for Se and Hg and with literature data (Table 4.1 and Table 4.2). The good agreement between measured and certified values confirms the reliability of the results obtained in this work.

Statistical Analysis

The concentrations of Se and Hg in µg Se / g and µg Hg / g were obtained by taking two replicates measurements and their average absolute deviation was calculated as follows :

-Average absolute deviation $= \frac{1}{n}\sum_{i=1}^{n}\left| x_i - \bar{x} \right|$

Where: x_i is the concentration; $\bar{x}$ is the mean concentration; and, n is the number of measurements.

CHAPTER FOUR

4.0 RESULTS

Table 4.1: Comparison of Certified values and Literature data with results obtained for Validation of CV-AAS method for the determination of Hg using DORM-2

Material	Concentration of Hg (μg g^{-1} dry $matter^{-1}$)			
	Measured	Certified	Literature	Reference for literature data
DORM-2	4.41 ± 0.11 [HG-AAS]	4.64 ± 0.26 [CVAAS; ID-ICP-MS]	4.55 ± 0.16 [CV-AFS]	Donkor et al.,(2006)

Data are presented as mean ± standard deviation

Table 4.2: Comparison of Certified values and Literature data with results obtained for Validation of HG-AAS method for Se determination using DORM-2

Material	Concentration of Se (μg g^{-1} dry $matter^{-1}$)			
	Measured	Certified	Literature	Reference for literature data
DORM-2	1.385 ± 0.06 [HG-AAS]	1.40 ± 0.09 [GFAAS; ID-ICP-MS]	1.390 ± 0.050 [HG-AAS]	Lavilla et al., (2007)
			1.370 ± 0.100 [HG-AAS]	Lavilla et al., (2008)

Data for measured concentration and literature values are presented as mean ± standard deviation for three replicate measurements

The concentrations of Se and Hg in the canned fish products are presented in Table 4.3. In all the 52 canned fish products (52 samples overall), the levels of Se and hence the molar concentrations of Se were higher than the corresponding levels of Hg and the molar concentrations of Hg.

Table 4.3: Se and Hg contents in the canned fish products

Fish product		Hg content		Se content	
Brand name	Code	µg Hg/ g [a]	µmol Hg kg^{-1}	µg Se/ g [b]	µmol Se kg^{-1}
Empress Mackerel	EM	0.182 ± 0.004	0.907 ± 0.019	0.364 ± 0.050	4.610 ± 0.633
Royal Boat Pilchards	RBP	0.145 ± 0.01	0.723 ± 0.050	0.243 ± 0.014	3.078 ± 0.177
Star Mackerel	SM	0.230 ± 0.02	1.147 ± 0.100	0.590 ± 0.090	7.472 ± 1.140
African Queen Mackerel	AQM	0.443 ± 0.022	2.208 ± 0.110	0.703.± 0.039	8.903 ± 0.494
Obaapapa Sardines	OS	0.082 ± 0.010	0.409 ± 0.500	0.105 ± 0.022	1.330 ± 0.279
Kaakyire Mackerel	KM	0.090 ± 0.02	0.449 ± 0.100	0.290 ± 0.062	3.673 ± 0.785
Star Kist Tuna	SKT	0.415 ± 0.045	2.069 ± 0.224	0.737±0.030	9.334 ± 0.380
Ohene Sardines	OHS	0.104 ± 0.01	0.518 ± 0.049	0.320±0.008	4.053 ± 0.101
Joly Omega-3 Sardines	JOS	0.253 ± 0.039	1.261 ± 0.194	0.401 ± 0.072	5.079 ± 0.912
Teacher Tuna Flake	TTF	0.364 ± 0.025	1.815 ± 0.125	0.449±0.005	5.686 ± 0.063
Vega Tuna Flake	VTF	0.500 ± 0.062	2.493 ± 0.309	0.875 ± 0.014	11.082 ± 0.177
Belma Sardines	BS	0.380 ± 0.004	1.894 ± 0.020	0.620 ± 0.080	7.852 ± 1.013
Titus Sardine	TS	0.314 ± 0.008	1.565 ± 0.040	0.368 ± 0.048	4.661 ± 0.608

[a, b] Data for µg Se/ g and µg Hg /g are presented as mean ± average absolute deviation for two replicate measurements

CHAPTHER FIVE

5.0 DISCUSSIONS

Levels of Se in the canned fish products ranges from 0.105 to 0.875 µg g^{-1}**.** There was significant variation in the canned fish products made from mackerel. The concentration of Se in AQM was 0.703 µg g^{-1} which was the highest and that of KM was 0.290 µg g^{-1} which was the lowest. There was a marked variation in the concentration of Se in the sardine products. The concentration of Se in BS was 0.620 µg g^{-1} which was the highest and the concentration of Se in OS was 0.105 µg g^{-1} which was the lowest among the sardine products.. The concentrations of Se in the other sardine products, TS (0.368 µg g^{-1}), JOS (0.401 µg g^{-1}) and OHS (0.320 µg g^{-1}) were at least three times more than that of OS. The concentration of Se in RBP was 0.243 µg g^{-1} which was more than twice that of OS .The concentrations of Se in VTF, SKT and TTF were 0.875 µg g^{-1}, 0.737 µg g^{-1} and 0.449 µg g^{-1} respectively.

There was significant variation in the Se concentrations in the canned fish products. The Se concentration of 0.105 µg g^{-1} for OS was the lowest among the 13 canned fish whiles the Se concentration of 0.875 µg g^{-1} for VTF was the highest. However, the Se concentration of 0.362 µg g^{-1} for EM and 0.368 µg g^{-1} for TS were almost the same. The Se concentration of 0.703 µg g^{-1} for AQM was almost the same as 0.737 µg g^{-1} for SKT. Hg contents in the canned fish products ranges from 0.082 to 0.500 µg g^{-1}. There was significant variation in the Hg concentrations in the mackerel products.

The Hg concentration of 0.443 for AQM was significantly higher than the Hg concentrations of 0.230 µg g^{-1} for SM, 0.090 µg g^{-1} for KM and 0.182 µg g^{-1} for EM for other mackerel products. The Hg concentration of 0.090 µg g^{-1} for KM was the lowest. The Hg concentration of 0.230 µg g^{-1} for SM was almost twice that of EM (0.182 µg g^{-1}). The sardine products also showed significant variation in their Hg concentrations. The Hg concentration of 0.380 µg g^{-1} for BS was the highest and 0.082 µg g^{-1} for OS was the lowest. The Hg concentration in RBP was 0.145 µg g^{-1}. The tuna products also showed variation in their Hg concentration. The Hg concentration of 0.500 µg g^{-1} for VTF was the highest among the tuna products and 0.364 µg g^{-1} for TTF was the lowest. The same trend was also observed in their Se concentrations.

In all the canned fish products, the Hg concentration of 0.082 µg g^{-1} for OS was the lowest and 0.500 µg g^{-1} for VTF was the highest. There was significant variation in the Hg concentrations in the fish products. However, KM and OS showed no significant variation in their Hg concentrations. The Hg concentration of 0.090 µg g^{-1} for KM and 0.082 µg g^{-1} for OS were almost the same. The Hg concentration of 0.443 µg g^{-1} for AQM and 0.415 µg g^{-1} for SKT were almost the same. The same trend was observed in their Se concentrations. The Hg concentration of 0.380 µg g^{-1} for BS and 0.364 µg g^{-1} for TTF were almost the same. The Hg concentration of 0.230 µg g^{-1} for SM and 0.253 µg g^{-1} for JOS were nearly the same. There was no significant variation in the Hg levels of 0.182 µg g^{-1} and 0.145 µg g^{-1} for EM and RBP respectively.

5.2 Molar concentrations of Se and Hg

Generally, in all the 13 canned fish products, the molar concentrations of Se were significantly higher than the corresponding molar concentration of Hg.

The molar concentrations of Se ranges from 1.330 to 11.082 μmol kg^{-1}. Molar concentrations of Hg in all the thirteen canned fish products were in the range 0.409 – 2.493 μmol kg^{-1}. There was significant variation in the molar concentration of Se in the fish products made from mackerel. The molar concentration of Se in the mackerel products were 4.6 μmol kg^{-1} for EM, 7.5 μmol kg^{-1} for SM, 8.5 μmol kg^{-1} for AQM, and 3.7 μmol kg^{-1} for KM. The Se molar concentration of 8.9 μmol kg^{-1} for AQM was more than twice the Se molar concentration of 3.7 μmol kg^{-1} for KM.

There was significant variation in the molar concentration of Se in the fish products made from tuna. The molar concentration of Se in VTF was 11.1 μmol kg^{-1}, SKT was 9.3 μmol $kg^{-1}$and TTF was 5.7 μmol kg^{-1}. The same trend was also observed in their molar concentration of Hg, that is 2.5 μmol kg^{-1} (VTF), 2.1 μmol kg^{-1} (SKT) and 1.8 μmol kg^{-1} (TTF). There was no variation in the molar concentrations of Se for OHS and TS.

The molar concentration of 4.1 μmol kg^{-1} for OHS was nearly the same as the molar concentration of 4.7 μmol kg^{-1} for TS. However, OS (Obaapapa Sardines), JOS (Joly Omega-3 Sardines) and BS (Belma Sardines) showed marked variation in their molar concentrations of Se. The molar concentration of 7.9 μmol kg^{-1} for BS was higher than the Se molar concentration of the other products (1.3 μmol kg^{-1} for OS, 4.1 μmol kg^{-1} for OHS, 4.7 μmol kg^{-1} for TS 5.1 μmol kg^{-1} for JOS). The Se molar concentration of BS (7.9 μmol kg^{-1}) was about six times that of OS (1.3 μmol kg^{-1}).

The Se molar concentration of 4.1 μmol kg^{-1} for OHS, 5.1 μmol kg^{-1} for JOS and 4.7 μmol kg^{-1} for TS were more than three times that of OS (1.3 μmol kg^{-1}).

5.3 Molar concentrations of Hg (μmol Hg kg^{-1})

There was wide variation in the molar concentrations of Hg for the canned fish products. The Hg molar concentration of 2.2 μmol kg^{-1} for AQM was the highest among the canned fish products made from mackerel and the other mackerel products, SM, EM, and KM were 1.1 μmol kg^{-1}, 0.9 μmol kg^{-1} and 0.4 μmol kg^{-1} respectively . The same trend was also observed in their Se molar concentrations. The Hg molar concentration of 2.2 μmol kg^{-1} for AQM was twice that of SM (1.1 μmol kg^{-1}). The Hg molar concentration of 0.4 μmol kg^{-1} for KM was the lowest among the mackerel products. The Hg molar concentration of AQM was at least five times more than that of KM. There was no significant variation in the Hg molar concentration in the fish products made from tuna. The molar concentration of Hg in VTF was 2.5 μmol kg^{-1}, and SKT was 2.1 μmol kg^{-1} whiles TTF was 1.8 μmol kg^{-1}. The Hg molar concentrations of OS and OHS were quite low compared to the other sardines products. The Hg molar concentration of 1.9 μmol kg^{-1} for BS was the highest among the fish products made from sardines and 0.4 μmol kg^{-1} for OS was the lowest. There was no significant variation in the Hg molar concentration of OS (0.4 μmol kg^{-1}) and OHS (0.5 μmol kg^{-1}).

5.4 Comparison between molar concentrations of Se (μmol kg^{-1})

There was significant variation in the molar concentration of Se in the canned fish made from mackerel and tuna (Fig 4.1).

The Se molar concentration of 11.1 μmol kg^{-1} for VTF was three times that of KM (3.7 μmol kg^{-1}). However, AQM and SKT showed slight variation in their molar concentrations of Se. AQM had the Se molar concentration of 8.9 μmol kg^{-1} whiles SKT was 9.3 μmol kg^{-1} which were nearly the same. EM, OHS and TS showed no significant variation in their molar concentration of Se.

The Se molar concentration of 4.6 μmol kg^{-1} for EM, 4.7 μmol kg^{-1} for TS and 4.1 μmol kg^{-1} for OHS were nearly the same. SM had Se molar concentration of 7.5 μmol kg^{-1} which was very close to 7.9 μmol kg^{-1} for BS. The Se molar concentration in OS was 1.3 μmol kg^{-1} which was significantly lower than the other products made from mackerel whiles the Se molar concentration of 8.9 μmol kg^{-1} for AQM was the highest. All the canned fish products made from mackerel had their Se molar concentration greater than that of RBP. However, there was slight variation in the Se molar concentration of KM and RBP (Fig 4.1). The Se molar concentration for KM was 3.7 μmol kg^{-1} which was very close to 3.1 μmol kg^{-1} for RBP. TTF and JOS showed slight variation in their molar concentration of Se. The Se molar concentration of 5.7 μmol kg^{-1} for TTF was almost the same as 5.1 μmol kg^{-1} for JOS. The Se molar concentration of SKT and VTF were greater than the other sardine products. The Se molar concentration of 1.3 μmol kg^{-1} for OS was quite low. VTF had Se molar concentration of 11.1 μmol kg^{-1} which was significantly higher than Se molar concentration of 1.3 μmol kg^{-1} for OS. The Se molar concentration of 3.1 μmol kg^{-1} for RBP was significantly lower than the canned fish products made from tuna. There was significant variation in the Se molar concentration of fish products made from tuna and RBP.

SKT had the Se molar concentration of 9.3 μmol kg^{-1} which was three times that of RBP (3.1 μmol kg^{-1}) and VTF was 11.1 μmol kg^{-1} which was nearly four times that of RBP (Fig 4.1). Apart from OS which had the lowest Se molar concentration among the fish products made from sardines and RBP, the other sardines products had their Se molar concentrations greater than that of RBP. BS had the Se molar concentration of 7.9 μmol kg^{-1} which was more than twice that of RBP (3.1 μmol kg^{-1}).

5.5 Comparison between molar concentrations of Hg (μmol kg^{-1})

Generally, in all the 13 canned fish products, the Hg molar concentrations were lower than the Se as shown in Fig 4.1. AQM, SKT and VTF showed no marked variation in their Hg molar concentrations. The Hg molar concentration of 2.5 μmol kg^{-1} for VTF, 2.1 μmol kg^{-1} for SKT and 2.2 μmol kg^{-1} for AQM were almost the same. However, the Hg molar concentration of 0.4 μmol kg^{-1} for KM was significantly low compared to the other fish products made from tuna. The Hg molar concentration of 2.5 μmol kg^{-1} for VTF was six times more than that of KM (0.4 μmol kg^{-1}).

The Hg molar concentration of KM and OS were the same, KM was 0.4 μmol kg^{-1} and OHS was 0.4 μmol kg^{-1}. OHS had Hg molar concentration of 0.5 μmol kg^{-1} and KM was 0.4 μmol kg^{-1} which were almost the same. In the canned fish products made from mackerel and sardine, AQM had the highest Hg molar concentration whiles KM and OS had the lowest (Fig 4.1).

SM and JOS also showed no significant variation in their Hg molar concentrations. The Hg molar concentration of 1.1 μmol kg^{-1} for SM was nearly the same as 1.3 μmol kg^{-1} for JOS. Apart from KM which had the lowest Hg molar concentration among the fish products made from mackerel and RBP, the other mackerel products had Hg molar concentration greater than that of RBP. The Hg molar concentration of AQM was almost three times that of RBP. AQM had Hg molar concentration of 2.2 μmol kg^{-1} whiles RBP was 0.7 μmol kg^{-1}. However, the Hg molar concentration of 0.7 μmol kg^{-1} for RBP was almost twice that of KM (0.4 μmol kg^{-1}). Generally, the Hg molar concentrations of canned fish products made from tuna were relatively higher than those of the sardine products.

However, TTF and BS had almost the same Hg molar concentration. The Hg molar concentration of 1.8 μmol kg^{-1} for TTF and 1.9 μmol kg^{-1} for BS showed no significant variation. VTF had the highest Hg molar concentration of 2.5 μmol kg^{-1} whiles OS (0.4 μmol kg^{-1}) was the lowest among the fish products made from tuna and sardine.

SKT had Hg molar concentration of 2.1 μmol kg^{-1} which was three times that of RBP (0.7 μmol kg^{-1}). In the canned fish products made from tuna and RBP, VTF had the highest Hg molar concentration whiles the RBP had the lowest. There was no significant variation in the Hg molar concentrations of RBP, OS and OHS. RBP had Hg molar concentration of 0.7 μmol kg^{-1}, OHS was 0.5 μmol kg^{-1} whiles OS was 0.4 μmol kg^{-1}. Two of the sardine products, BS and TS had their Hg molar concentration at least twice that of RBP.

5.5 Molar ratios of Se : Hg and Hg : Se

In all the 13 canned fish products, the Se : Hg molar ratios were higher than the corresponding Hg : Se molar ratios (Fig 4.2 and Fig 4.3). The Se : Hg molar ratio ranges from 2.977 to 8.186 and Hg : Se molar ratio were in the range 0.122 – 0.336.

5.5.0 Se : Hg molar ratios

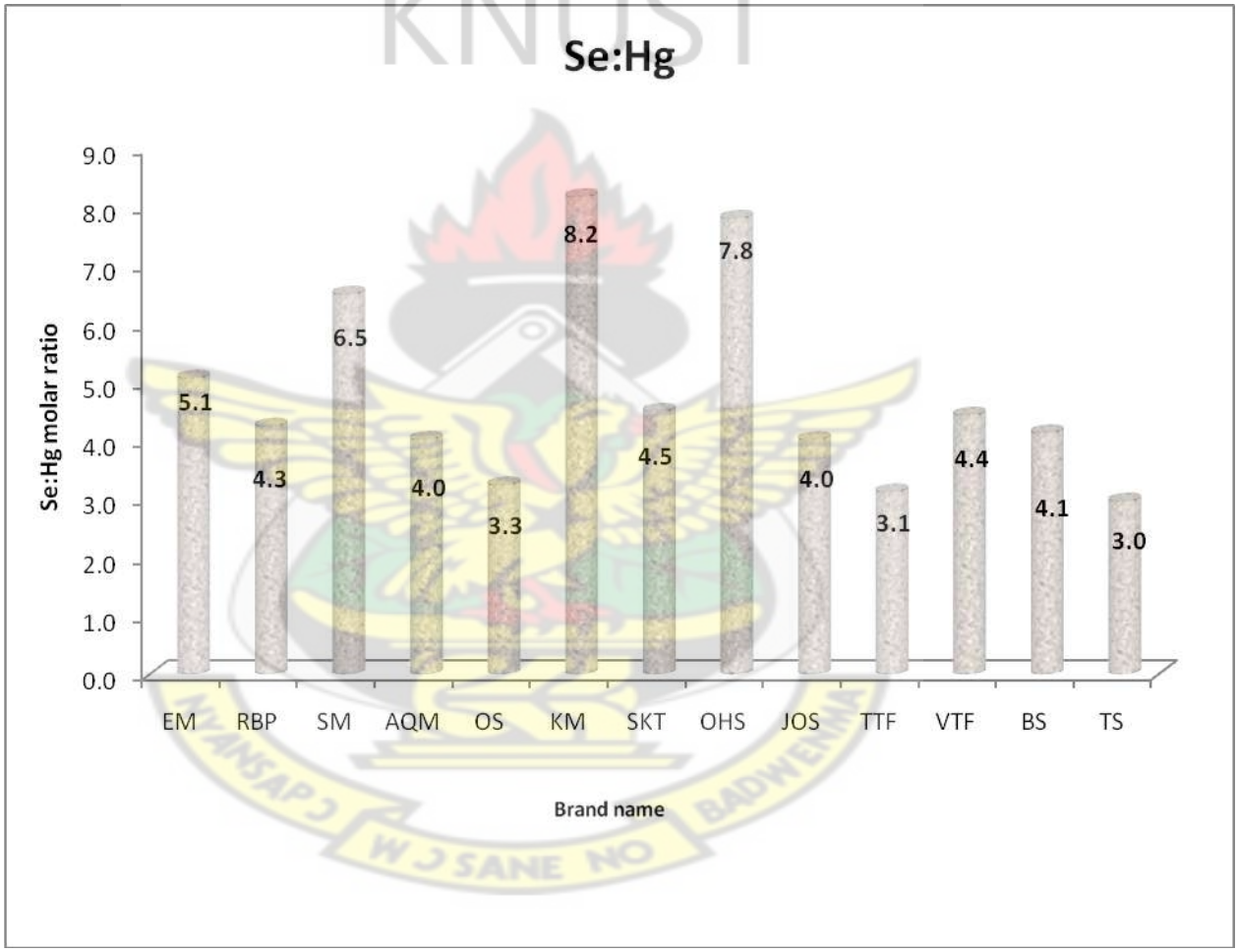

Fig. 5.1 Se: Hg molar ratios in the individual canned fish products (Appendix 1)

For the canned fish products made from mackerel, KM had the highest Se: Hg ratio and AQM had the lowest as depicted on Fig 4.2.

There was significant variation in the Se: Hg molar ratio in the fish products made from mackerel. The Se : Hg molar ratio for KM was 8.2, SM was 6.5, EM was 5.1 and AQM was 4.0.

Generally, for the canned fish made from tuna, SKT and VTF had almost the same Se: Hg ratios. SKT had Se: Hg ratio of 4.5 and VTF was 4.4 whiles TTF was 3.1. SKT had the highest Se: Hg (4.5) whiles TTF was the lowest (3.1).

In the canned fish products made from sardine, OS and TS had almost the same Se: Hg molar ratios (Fig 4.2). The Se: Hg ratio in OS was 3.3 and TS was 3.0. There was no significant variation of Se: Hg values in JOS (4.0) and BS (4 .1). However, the Se: Hg molar ratio for AQM was significantly higher than the other sardines. TS had the lowest Se: Hg ratios (Fig 4.2).

There was no significant variation in the Se: Hg ratios for SKT, and VTF and recorded Se: Hg ratios of 4.5 and 4.4 respectively. KM had the highest Se: Hg ratio and TTF with the lowest (Fig 4.2). With the exception of AQM, other mackerel products had Se: Hg ratio greater than canned fish products made from tuna. The Se: Hg ratios of 4.0 for AQM and 4.0 for JOS were the same. Also there was no marked difference in Se: Hg ratio of JOS, BS and AQM. The Se: Hg ratio of 4.0 for JOS, 4.1for BS and 4.0 for AQM were nearly the same. However KM had the highest Se: Hg ratio of 8.2 followed by OHS (7.8) with Se: Hg ratio greater than other sardine products. RBP and AQM had almost the same Se: Hg ratio. RBP had Se: Hg ratio of 4.3 and 4.0 Se: Hg ratio for AQM showed no significant variation. However, EM, SM, KM had their Se: Hg ratios greater than RBP.

Generally, there was no significant variation in the Se: Hg molar ratio of SKT, VTF, JOS and BS (Fig 4.2). The Se: Hg molar ratio of 4.5 for SKT, 4.4 for VTF, 4.1 for BS and 4.0 for JOS were nearly the same. OS, TS, and TTF also showed no significant variation in their Se: Hg molar ratios. OS had 3.3 Se: Hg molar ratio, TS was 3.0 and TTF was 3.1 which were almost the same. However, OHS had the Se: Hg molar ratio of 7.8 which was significantly higher than the other products made from tuna and sardine. There was no significant variation in the Se: Hg molar ratio of SKT, VTF and RBP. The Se: Hg molar ratio of 4.5 for SKT, 4.4 for VTF and 4.3 for RBP were almost the same.

There was no significant variation in the Se: Hg molar ratio of RBP, JOS and BS. RBP had the Se: Hg molar ratio of 4.3 which was almost the same as the Se: Hg molar ratio of 4.0 for JOS and 4.1 for BS. However, the Se: Hg molar ratio of 7.8 for OHS was almost twice the Se: Hg molar ratio of 4.3 for RBP.

5.5.2 Hg : Se Molar ratios

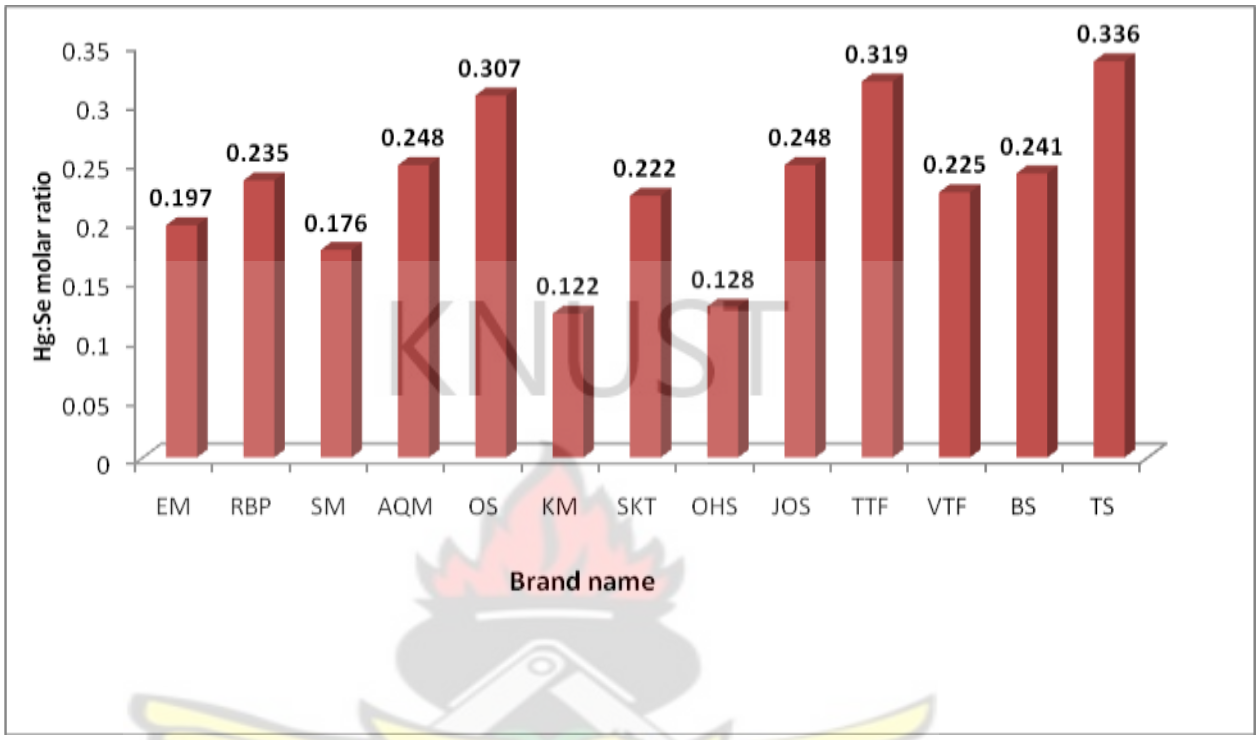

Fig. 5.2 Hg: Se molar ratios in individual canned fish product

Generally, for the canned fish products made from mackerel, EM and SM had almost the same Hg: Se molar ratio, however, the Hg: Se molar ratio of AQM (0.248) was significantly higher than the other mackerel products (Fig 4.3). The detailed results are presented in Appendix 1.

The Hg: Se molar ratio of AQM was at least twice that of KM. KM had the lowest Hg: Se molar ratio (Fig 4.3). There was no marked difference in the Hg: Se molar ratios of EM and SM. The Hg: Se ratio of 0.197 for EM was almost the same as Hg: Se ratio of 0.176 for SM.

For the canned fish products made from Tuna, SKT and VTF had almost the same Hg: Se molar ratios as shown in Fig 4.3. However, the Hg: Se ratio of 0.319 for TTF was significantly higher than the other tuna products. The Hg: Se ratio of 0.222 for SKT and 0.225 for VTF showed no marked difference. SKT had the lowest Hg: Se molar ratio.

In the canned fish products made from sardine, JOS and BS showed no significant variation. The Hg: Se molar ratios of 0.248 for JOS and 0.241 for BS were almost the same. This trend was also observed in their Se: Hg molar ratios. There was slight variation in the Hg: Se molar ratio of OS and TS. OS had 0.307 Hg: Se molar ratio which was very close to the Hg: Se molar ratio of 0.336 for TS. This trend was also observed in their Se: Hg molar ratios. TS had the highest Hg: Se molar ratio among the canned fish products made from sardine. OHS had the lowest Hg: Se molar ratio. The Hg: Se molar ratio of OHS was quite low compare to the other canned fish products made from sardine. OS, JOS, BS and TS had almost twice the Hg: Se molar ratio of OHS (Fig 4.3).

There was no significant variation in the Hg: Se molar ratios of AQM, SKT and VTF. The Hg: Se molar ratio of 0.248 for AQM, 0.222 for SKT and 0.225 for VTF were almost the same. Relatively, the Hg: Se molar ratios of the canned fish products made from mackerel were quite low compare to the Hg: Se molar ratios of tuna products. Among the canned fish products made from mackerel and tuna, TTF had the highest Hg: Se molar ratio (0.319) and KM was the lowest (0.122).

There was no significant variation in the Hg: Se molar ratios of AQM, JOS and BS. AQM and JOS had the same Hg: Se molar ratio of 0.248. BS had Hg: Se molar ratio of 0.241 which was almost the same as the Hg: Se molar ratio of 0.248 for AQM and JOS.

KM and OHS showed no significant variation in their Hg: Se molar ratios. KM had Hg: Se molar ratio of 0.122 which was very close to 0.128 Hg: Se molar ratio for OHS. Among the canned fish products made from mackerel and sardine, TS had the highest Hg: Se molar ratio and KM had the least Hg: Se molar ratio.

RBP and AQM had Hg: Se ratios which were almost the same. The Hg: Se ratio for AQM was 0.248 and that of RBP was 0.235 which showed no significant variation. Apart from the AQM, the other mackerel products had Hg: Se ratios Hg: Se ratios less than Hg: Se ratio of RBP. There was no significant variation in the Hg: Se ratio of SKT, VTF, JOS and BS. The Hg: Se ratio of 0.222 for SKT, 0.225 for VTF, 0.248 for JOS and 0.241 for BS showed no marked difference. TTF, OS and TS showed no significant variation in their Hg: Se ratio. However, in the fish products made from tuna and sardine, OHS had 0.128 Hg: Se which was the lowest and TS had 0.336 Hg: Se ratio which was the highest. Generally, there was no significant variation in the Hg: Se ratio of SKT, VTF and RBP. RBP had almost twice the Hg: Se ratio as that of OHS. JOS, BS and RBP showed no significant variation in their Hg: Se ratio. TS had the highest Hg: Se ratio among the canned fish products made from sardine whiles OHS had the lowest.

5.6 Free selenium (µmol kg^{-1}) and Se Health Benefit Value (SeHBV) (µmol kg^{-1})

The Se was in excess over Hg in all the 13 canned fish products. The free Se values for all the fish products were positive. The SeHBVs were positive for all the fish products. The SeHBVs were higher than the corresponding free Se in all the canned fish products. The detailed results are presented in Appendix 1.

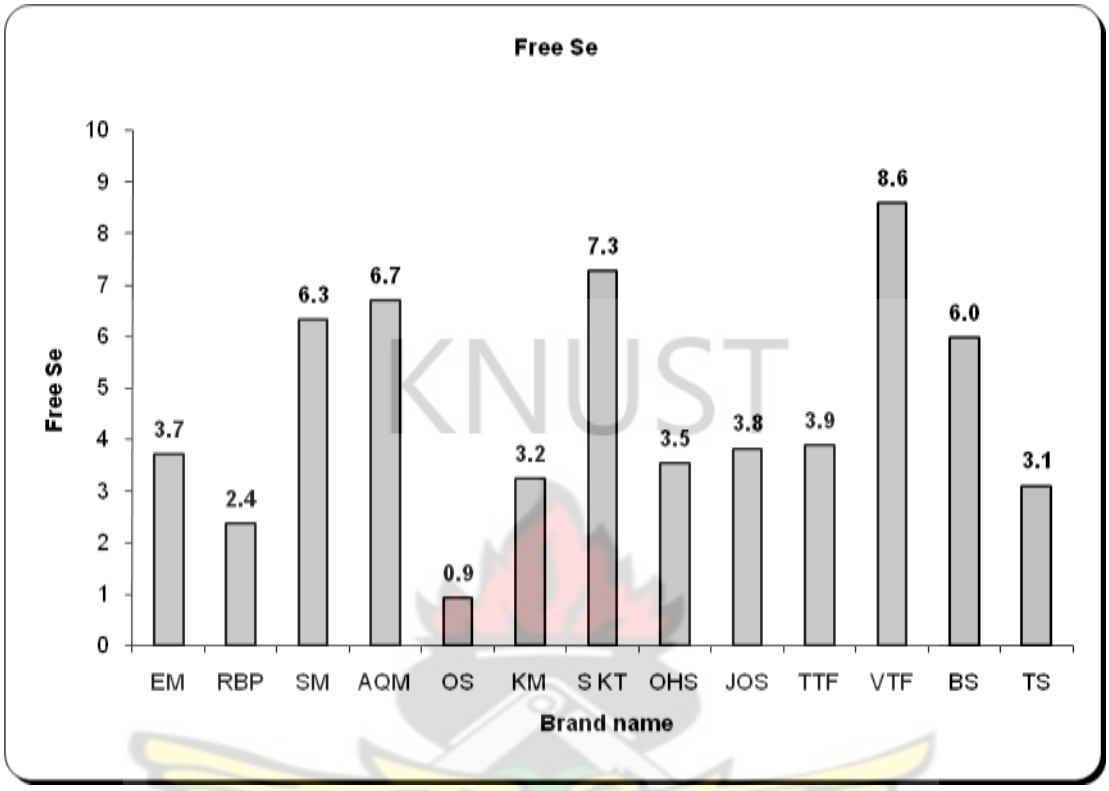

Fig 5.3 The free Se in the individual canned fish products

5.6.1 The Free Selenium

The free Se levels of the 13 canned fish ranges from 0.921 to 8.589 as depicted on Fig 4.4. SM and AQM showed no marked difference in the free Se values.

The free Se value of 6.325 for SM was almost the same as 6.695 free Se value for AQM. EM and KM had almost the same free Se value. Free Se value of EM was 3.703 and that of KM was 3.224 which showed no significant variation. However, SM and AQM had the free Se value almost two times that of the free Se value of EM and KM (Fig 4.4). AQM had the highest free Se value whiles KM had the lowest.

There was slight variation in the free Se values of VTF and SKT. However, the free Se value of 8.589 for VTF was about two times higher than free Se value of 3.871 for TTF. The free Se value for SKT was almost twice that of the free Se value of TTF and VTF had almost three times free Se value of TTF. Generally, for the canned fish products made from sardine, OHS, JOS and TS had almost the same free Se values. However, the free Se value of BS was significantly higher than the other sardines products. OHS, JOS, and TS had the free Se value almost three times the free Se value of OS.

EM, KM and TTF showed no marked difference in the free se values. The free Se value of 3.703 for EM, 3.224 for KM and 3.871 for TTF were almost the same. SKT and VTF had free Se values higher than the free Se values of the canned fish products made from mackerel. VTF had the highest free Se value whiles KM had the lowest. The free Se value of VTF was almost three times that of KM. OHS, JOS, TS, EM, and KM showed no significant variation in the free Se values. The free Se value of 3.535 for OHS, 3.818 for JOS and 3.096 for TS were almost the same as the free Se value of 3.703 for EM and 3.224 for KM. BS, SM and AQM showed slight variation in their free Se values. BS had free Se value of 5.958, SM had 6.325 free Se and AQM had 6.695 which were very close. Relatively, OS had the lowest free Se value (0.921) whiles AQM had the highest free Se value among the fish products made from mackerel and sardines. All the canned fish products made from mackerel had their free Se values greater than the RBP. The free Se values of SM (6.3) and AQM (6.7) were almost three times the free Se value of RBP (2.4). The variation in the free Se values of TTF, OHS, JOS and TS was not significant.

TTF had the free Se value of 3.871 which was almost the same as the free Se value of 3.818 for JOS, 3.535 for OHS and 3.096 for TS.

Apart from BS, the other fish products made from sardines had their free Se less than the free Se of the tuna products. VTF and SKT had the free Se value relatively higher among the fish products made from tuna and sardines. VTF had the highest free se and OS had the lowest in the canned fish made from tuna and sardines.

All the fish products made from tuna had their free Se value greater than the free Se of RBP. SKT had the free Se value as much as three times the free Se of RBP. VTF had almost four times OHS, JOS, BS and TS had free Se value greater than the free Se value of RBP. However, there was slight variation in the free Se value of OHS, JOS, TS and RBP. RBP had free Se value of 2.355 which was significantly higher than the free Se value of 0.921 for OS. The free Se value of 5.958 for BS was two times more than the free Se of RBP.

5.6.2 Se Health Benefit Value (SeHBV) (µmol kg^{-1})

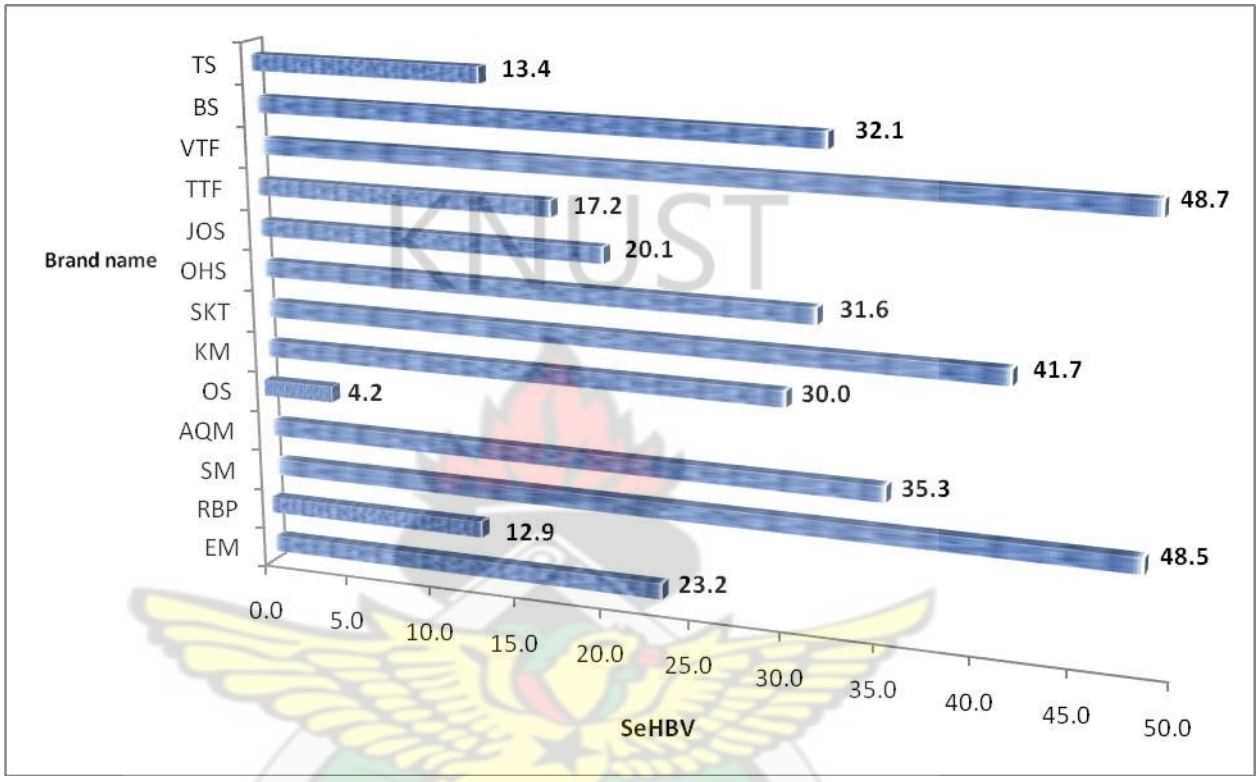

Fig. 5.4 Se health benefit value (SeHBV) for the canned fish products (appendix 1)

The SeHBV were in the range 4.201 – 48.710. There was marked difference in the Se HBV of canned fish made from mackerel. The Se HBV of SM was significantly higher than the other mackerel products. The Se HBV of 48.5 for SM was more than twice that of EM which was 23.2.

EM had the lowest Se HBV among the canned fish made from mackerel. AQM and KM had Se HBV of 35.3 and 30.0 respectively. In the tuna canned fish products, VTF had the highest Se HBV, followed by SKT and TTF had the lowest.

There was marked variation in the Se HBV of the tuna products (Fig 4.5). VTF had Se HBV of 48.7, SKT was 41.7 and TTF was 17.2. VTF and SKT had their Se HBV more than twice that of TTF. This trend was also observed in their Se and Hg molar concentrations as well as their free Se values. There was significant variation in the Se HBV of the fish products made from sardines, however, OHS and BS showed slight variation.OHS had Se HBV of 31.6 and BS had 32.1. The Se HBV of 4.2 for OS was quite low compare to the other fish products made from sardine. TS had Se HBV of 13.4 which was three times higher than that of OS (4.2). JOS had Se HBV of 20.1 which was almost five times that of OS whiles OHS (31.6) and BS (32.1) had almost eight times that of OS.

Among the fish products made from mackerel and tuna, VTF had the highest Se HBV whiles TTF had the lowest. There was no significant variation in the Se HBV of SM and VTF. SM had Se HBV of 48.5 which was almost the same as the Se HBV of 48.7 for VTF. AQM had Se HBV of 35.3 which was two times more than TTF which had 17.2. All the mackerel products had their Se HBV greater than that of TTF. The Se HBV of VTF was more than twice that of EM. Apart from SM, SKT had its Se HBV higher than the other mackerel products. In the canned fish products made from mackerel and sardine, SM had the highest Se HBV whiles OS had the lowest. SM had Se HBV of 48.5 and OS had 4.2 which showed a significant variation. The Se HBV of SM was eleven times more than that of OS. There was slight variation in the Se HBV of KM, BS and OHS (Fig 4.5). KM had Se HBV of 30.0, OHS had 31.6 whiles BS had 32.1.

The Se HBV of 23.2 for EM was five times more than that of OS. The Se HBV of SM was more than twice that of JOS. There was significant variation in the Se HBV of canned fish products made from mackerel and RPB. EM had Se HBV of 23.2 which was almost twice that of RBP which was12.9. KM had Se HBV of 30.0 which was two times greater than that of RBP (12.9). SM had the highest Se HBV among the mackerel and RBP had almost four times that of RBP. All the mackerel products had their Se HBV greater than the RBP. Apart from TTF, the other tuna products, SKT and VTF had their Se HBV significantly higher than Se HBV of sardine products. The Se HBV of 4.2 for OS was extremely low compare to the Se HBV of tuna products. SKT had Se HBV of 41.7 which was about ten times that of OS. SM had Se HBV of more than three times that of TS. The Se HBV of tuna products was significantly higher than the RBP. All the tuna products had their Se HBV greater than that of RBP.VTF had Se HBV of 48.7 which was about four times that of RBP (12.9). SKT had more than three times Se HBV of RBP. TS and RBP showed no significant variation in their Se HBV. TS had Se HBV of 13.4 which was almost the same as the Se HBV of 12.9 for RBP. The Se HBV of RBP was three times greater than that of OS. OHS and BS had more than twice Se HBV of RBP.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

The levels of Hg in the canned fish products were in the range 0.082 – 0.500 μg g^{-1}. The Se contents in the fish products ranges from 0.105 to 0.875 μg g^{-1}. The molar concentrations of Hg and Se were ranged from 0.409 to 2.493 μmol kg^{-1} and 1.330 to 11.082 μmol kg^{-1} respectively. Se:Hg molar ratio ranges from 2.977 to 8.186 and Hg:Se molar ratio were in the range of 0.122 – 0.336. The free Se levels of the 13 canned fish products ranged from 0.921 to 8.589 μmol kg^{-1}. The SeHBV ranges from 4.201 to 48.710 μmol kg^{-1}.

The Se:Hg molar ratio values were all greater than one (1), indicating that there was enough Se to protect against Hg toxicity.

The Hg:Se molar ratio values were all less than one (1), which shows that there is no Hg health risk associated with the consumption of the 52 canned fish products.

The SeHBV for all the canned fish products were positive. This implies that there is no Hg health risk associated with the consumption of the fish products.

6.2 RECOMMENDATIONS

Based on the research findings, the underlisted recommendations are made:

(a) Environmental activists and researchers must consider both Hg and Se contents in canned fish products before making conclusive statements on the Hg health risk associated with the consumption of canned fish products.

(b) In order to assure consumers of the safety of the fish products, Food and Drug Board (FDB) and Ghana Standard Authority (GSA) must demand mandatory assessment of the Se:Hg, Hg:Se, free Se and SeHBV by the canned fish producing industries. Such results should be boldly displayed on the products.

(c) Monitoring of canned fish products on the Ghanaian market based on their SeHBV to assess Hg toxicity should be continuous since new canned fish products keep emerging on the market.

(d) Further studies should be done on the fresh, dried and the canned fish of the same species to ascertain if processing can affect the Hg and Se contents in the fish as well as SeHBV.

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APPENDIX 1

Detailed results of Se-Hg and Hg-Se molar ratios, free Se, and SeHBV

Canned Fish	Code	Se:Hg	Hg:Se	Free Se	SeHBV
Empress Mackerel	EM	5.1	0.197	3.7	23.2
Royal Boat Pilchards	RBP	4.3	0.235	2.4	12.9
Star Mackerel	SM	6.5	0.176	6.3	48.5
African Queen Mackerel	AQM	4.0	0.248	6.7	35.3
Obaapapa Sardines	OS	3.3	0.307	0.9	4.2
Kaakyire Mackerel	KM	8.2	0.122	3.2	30.0
Star Kist Tuna	SKT	4.5	0.222	7.3	41.7
Ohene Sardines	OHS	7.8	0.128	3.5	31.6
Joly omega-3 Sardines	JOS	4.0	0.248	3.8	20.1
Teacher Tuna Flake	TTF	3.1	0.319	3.9	17.2
Vega Tuna Flake	VTF	4.4	0.225	8.6	48.7
Belma Sardines	BS	4.1	0.241	6.0	32.1
Titus Sardines	TS	3.0	0.336	3.1	13.3

APPENDIX 2

The Photograph of Thirteen Canned Fish Products used for the Study