ASSESSMENT OF THE LEVELS OF THIOCYANATE IN PROCESSED AND UNPROCESSSED RED AND BROWN FINGER MILLET (Eleusine coracana) GROWN IN MOGOTIO AREA, BARINGO COUNTY, KENYA

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NOVEMBER 2014
DECLARATION

I hereby declare that this is my original work and has not been submitted for the award of a degree in this or any other university.

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This thesis has been submitted with our approval as the university's supervisors.

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DEDICATION

I dedicate this work to my beloved husband Daniel Cherono and our children Ian Kipchumba and Dennis Kipkemboi and my parents David Chebon and Eunice Talaa.
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# ABBREVIATIONS AND ACRONYMS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>ATSDR</td>
<td>Agency for Toxic Substances and Disease Registry</td>
</tr>
<tr>
<td>C-GF</td>
<td>C-glycosyl Flavanol</td>
</tr>
<tr>
<td>CNGs</td>
<td>Cyanogenic Glycosides</td>
</tr>
<tr>
<td>FSA</td>
<td>Food Science and Agriculture</td>
</tr>
<tr>
<td>ICRISAT</td>
<td>International Crops Research Institute for the Semi-arid tropics</td>
</tr>
<tr>
<td>Glgs</td>
<td>Glucosinolates</td>
</tr>
<tr>
<td>IDD</td>
<td>Iodine Deficiency Disorders</td>
</tr>
<tr>
<td>LED</td>
<td>Light-Emitting Diode</td>
</tr>
<tr>
<td>PVS</td>
<td>Participation Varietal Show</td>
</tr>
<tr>
<td>TGR</td>
<td>Total Goiter Rate</td>
</tr>
<tr>
<td>TPO</td>
<td>Thyroid Peroxidase</td>
</tr>
<tr>
<td>NIS</td>
<td>Sodium-Iodide Symporter</td>
</tr>
<tr>
<td>SNK</td>
<td>Student Newman Keul's</td>
</tr>
<tr>
<td>UV-VIS</td>
<td>Ultraviolet-Visible Spectroscopy</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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Finger millet (*Eleusine coracana*) is an important African staple food crop in the tropics. The crop contains cyanogenic glycosides which can be readily converted to thiocyanate by glycosidases and sulfur transferase enzymes present in the plant or in the animal tissues. Thiocyanate inhibits the uptake of iodine by the iodide pump of the thyroid gland thus acting as a goitrogen which suppresses thyroid function leading to goiter. Goiter increases the risk of thyroid irradiation in the event of a nuclear accident and hypothyroidism during pregnancy which may cause minor brain damage of offspring. In 1994 the National goiter prevalence in Kenya was 16.3%. Survey data from Rift Valley where Mogotio is situated indicated total goiter prevalence around 20%. Research has associated millet diets with goiter although the thiocyanate content of finger millet is still unknown. Finger Millet is edible as whole grain or cooked. The dry grains can be processed prior to cooking by soaking, sprouting and fermentation. The green and the dry leaves are used as animal feed so that the whole crop is utilized. The red and the brown varieties of finger millet are commonly grown in Mogotio. It was therefore important to determine the level of thiocyanate in processed and unprocessed finger millet in the region to obtain information that will be precautionary in addressing goiter. The levels of thiocyanate were analysed in the dry, sprouted and soaked grains, fresh, fermented and cooked flour. The green and dry leaves of the plant were also analysed for thiocyanate. Samples of the red and brown varieties of finger millet were randomly selected from the farmers in the area. Thiocyanate content was analysed using UV-VIS spectrophotometric detection. The data was analysed using ANOVA and independent T-test. Separation of means was done by SNK test. Levels of thiocyanate content in the red finger millet ranged from 43.48±1.56 to 4.28±0.5 mg/kg with the fresh dried grains having the highest followed by sprouted grains and cooked flour had the lowest levels. For the brown finger millet levels ranged from 53.30±0.78 to 4.96±0.40 mg/kg, with sprouted grains having the highest levels while cooked flour had the least amount. Green leaves had 31.69±0.71 and dried leaves had 8.80±0.14 mg/kg. The results indicated that, there were significant differences between the levels that were found in the grains and the flour (p< 0.001). The amounts measured in the current research indicate that the thiocyanate content found in the finger millet samples were within the recommended levels (100 mg/kg) but the frequency of ingestion may still result into health risks. It is therefore advocated that processing prior to cooking be encouraged as this reduces thiocyanate levels.
CHAPTER ONE
INTRODUCTION

1.1 Background information

Goiter is a swelling on the thyroid gland. Its development is critically related to the balance between iodine and thiocyanate, a goitrogen found in some African diets (Toure et al., 2003). More than 5% of the world’s populations have goiters. Many of these are associated with diverse disorders and constitute a major public health problem (Adwok, 2006). Although 300 million people with goiter live in less highly developed countries where iodine deficiency is prevalent, 100 million individuals with goiter live in more highly developed countries where goiter continues to occur in certain areas, despite iodine prophylaxis (Gaitan, 1990). In Africa goiter is endemic in many countries notably Congo, Uganda, Kenya and Sudan, the prevalence is as high as 81% in some parts of these countries (Elnour et al., 2000).

The most common worldwide cause of goiter is iodine deficiency, usually seen in countries that do not use iodized salt. Selenium deficiency is also considered a contributing factor. In countries that use iodized salt, Hashimoto’s thyroiditis is the most common cause (Hashemipour et al., 2007). Iodine deficiency disorder (IDD) is widespread in mountainous and sub- mountainous as well as flood plains and sandy leached soil tracts of the world (Narwal, 2013). The commonly quoted historical areas of goiter include the Himalayas slopes, the Andean region of South America, the European Alps and the mountainous areas of China. The Rift Valley regions of East and Central Africa where Mogotio is situated are also endemic areas (Adwok, 2006). A National
micronutrient survey conducted in 45 districts in Kenya found total goiter rates (TGR) as 16.3% (FAO, 2005). Three had no goiter problems, while 30 had mild IDD with goiter prevalence between 5 and 19%, 7 had TGR as (20-29%) and 5 districts were noted to have goiter prevalence’s greater than 30%. Survey data from Rift Valley where Mogotio is situated indicated total goiter prevalence as 20% (FAO, 2005). Cases of goiter have been reported in Mogotio despite the use of iodized salt (Adwok, 2006). Doctors who carried out goiter surgical operation services in three towns of the north Rift region including Kitale, Kapenguria and Lodwar reported that the disease is highly prevalent (Wamalwa, 2012).

Goiter increases the risk of thyroid irradiation and hypothyroidism during pregnancy and early infancy (with a concomitant risk of minor brain damage and irreversible impairment of the neuro psycho intellectual development of offspring (Hashemipour et al., 2007). The enlarged thyroid compresses the trachea and oesophagus leading to symptoms such as coughing, breathing difficulties, hoarseness and swallowing difficulties (Norman, 2011).

Despite sufficient iodine intake, causes other than iodine deficiency, like unknown goitrogens, protein-energy malnutrition, vitamin A, iron, selenium and zinc deficiencies or their combination, may be responsible causes (Hashemipour et al., 2007). The global salt iodization program has resulted in a reduction in goiter prevalence. The persistence of goiter in some areas with adequate iodine prophylaxis suggests the existence of other goitrogenic factors. Goitrogens are chemicals that are ingested in foods or drugs.
Examples include the cyanogenic glycosides and thiocyanate found in some plants. These chemicals can suppress thyroid function in different ways (Marcelle, 2011). Some goitrogens induce antibodies that cross-react with the thyroid; while others interfere with thyroid peroxidase (TPO), the enzyme responsible for adding iodine during production of thyroid hormones (Dasgupta, 2008). Cyanogenic glycosides are naturally occurring goitrogens found in staple foods in the tropics, namely cassava, millet, sorghum, maize, bamboo shoots, beans and sweet potatoes (Adwok, 2006).

Millet (Eleusine, Pennisetum, Setaria, Echinoloa and Paspalum) is a source of thiocyanate whose goitrogenic effects are additive to those of the C-glycosyl flavanol (C-GF) (Makokha, 2002). Thiocyanate and isothiocyanate have been demonstrated as the goitrogenic principles of cyanogenic plants (Chandra et al., 2004). Millet and sorghum are sources of dhurrin which upon hydrolysis yield cyanide, a sugar and a ketone or aldehyde (Saidu, 2004). After ingestion, these glycosides can be readily converted to thiocyanate by widespread glycosidases and the sulfur transferase enzyme (Chandra et al., 2004). The highly potent thiocyanate is implicated in the high cases of goiter in millet and cassava eating population (Toure et al., 2003). Consumption of pearl millet is considered one of the factors responsible for high incidence of goiter in rural populations (Gaitan, 1989). In Sri Lanka the goitrogenic effect of the commonly used finger millet (Eleusine Coracana) were attributed to three types of C-GF. Epidemiological evidence suggests that millet might play a role in the etiology of endemic goiter. In Sudan a traditional fermentation procedure of two pearl millet cultivars grown in the area modified their effects on the weight of the thyroid gland and thyroid hormone profile in
rats (Elnour et al., 2000). Millet’s goitrogenic agent is apparently associated with the bran and endosperm fractions and might be related to the grains high content of minerals (Klopfenstein et al., 2012). Besides, the crop is a major source of energy, protein, vitamins and minerals such as potassium, iron, zinc, copper and manganese (Ocheme, 2007).

Thiocyanate ion acts as a goitrogen when present at high concentration especially when the iodine content of the diet is low (Malik et al., 2012). Studies by Chandra et al. (2004) and Malik et al. (2012) reported the levels of thiocyanate (mg/kg) in cassava (12.95), cabbage (23), cauliflower (42.3), mustard (50.5), kale (159), sweet potatoes (20.5) and carrots (16.5). Gaitan et al. (1989) reported the levels of thiocyanate in pearl millet (35 mg/kg) and Chweya (1990) determined the content of thiocyanate in the leaves, shoots and petioles of kales from various regions. Thiocyanate content was described as high in these plants and this observation suggest that in addition to iodine deficiency, dietary intake of cyanogenic plant having high thiocyanate content may play some role for the persistence of endemic goiter during the post iodization period (Malik et al., 2012). The concentration of thiocyanate (SCN⁻) required for half-maximal stimulation of the thyroid function is much lower (0.5- 1 μM) than that required for the inhibition (60-80 μM). The normal level of thiocyanate in blood serum should not exceed 100 mg/L (WHO, 2003). Thiocyanate or isothiocyanate like compounds primarily inhibit the iodine concentrating mechanism of the thyroid, and their goitrogenic activity can be overcome by iodine administration. In higher concentrations thiocyanate also inhibits thyroid hormone synthesis by interfering with thyroid
peroxidase (TPO) (Chandra et al., 2004). Thiocyanates have been found to block the sodium–iodide symporter (NIS) and prevent uptake of iodine into the thyroid gland (Sanchez, 1996). As a consequence, SCN$^-$ ultimately results in an Iodine-deficient thyroid and a decrease in thyroid hormone synthesis (Contempre' et al., 2004). When the diet is overly rich in goitrogens, the thyroid gland swells to trap as much iodine as possible forming a goiter or a lump in the neck. Studies have shown that thiocyanate binds to the same regulatory site as iodine but with a slightly lower affinity (Virion et al., 2005). Thiocyanate ion has a molecular volume and charge similar to that of iodide and because of the similarities in chemical properties with the halides it is termed as a pseudo-halide (Virion et al., 2005). Studies have also identified other nutritional inhibitors and toxic substances associated with millet grains, these include cyanide (cyanogenic glycosides), polyphenols, phytates, tannins, oxalic and phytic acid (Le'der, 2004). Several factors like storage conditions affect the nutritional quality of millet and being a staple consumed at household levels processing must be considered. Furthermore environmental factors such as seasonal variation, pests and diseases may increase thiocyanate (Chandra et al., 2004).

Millet can be processed prior to cooking by drying, soaking, sprouting and fermentation (Ikemefuna, 1994). Dried grains are usually ground to fresh flour which is used directly to make thin or thick porridge (cooked flour). Alternatively, fresh flour can be fermented prior to cooking. Steeping of cereal grains in water followed by sprouting or germination is a common household practice in developing countries including Kenya (Chove and Mamiro, 2010). Sprouting reduces the high viscosity and water-binding characteristic of
starch based porridges. As soon as the grain seeds are hydrated, changes occur, which result in partial breakdown of storage components, such as starch and proteins. The starch nature of the un-sprouted grains allows these foods to bind so much water yielding a thick porridge (Onyeka and Dibia, 2002). Dilution of the porridge increases bulk and renders the food more difficult for infants to consume in one sitting. The bulkiness of the porridge limits adequate amount of nutrient intake by the infants. Thus sprouting is mainly used to lower dietary bulk in grains because it converts significant amounts of starch, which is principally responsible for the viscosity in grain gruels, to sugars and short chain oligosaccharides. These methods of processing affect thiocyanate levels (Traoré et al., 2004).

Soaking normally precedes cooking and fermentation, it provides a suitably larger medium for fermentation and allows for greater extraction of the soluble thiocyanate into the soaking water. Prolonged soaking in water effect the breakdown of tissue and extraction of the starchy mass. A simulation of the method followed by drying showed a reduction of cyanide of about 98.6% of the initial content (Ayenor, 1985). Free thiocyanate is rapidly lost in boiling water. About 90% of free thiocyanate is removed within 15 minutes of boiling compared to 55% reduction in bound thiocyanate after 25 minutes (Tewe, 2003). Microwaving crucifers reduces thiocyanate yield to one half, steaming them reduces this yield to one third, boiling them for half an hour and dumping out the water almost eliminates this yield (Master, 2008). A study on cyanide content of *E.coracana* showed that sprouting the grains significantly increased the levels more than
two-fold (by factors ranging between 2.11 and 2.14) from raw to sprouting stage (Chove and Mamiro, 2010).

Finger millet is the second most important food crop in Mogotio after maize. Mogotio lies in the lower end of Baringo County at 900-1000 m above sea level. The area is predominantly semi-arid land. Annual rainfall in this zone varies from 400 to 750 mm. The low and uncertain rainfall restricts successful dry land cropping to drought resistant crops such as *E. coracana* and sorghum (Peter, 1992). Finger millet varieties vary in their grain colours from, white, orange, red, brown, purple to almost black, but farmers in the area cultivate the red and the brown varieties. Due to serious droughts, International crops research institute for the semi-arid tropics (ICRISAT) together with Egerton University have come up with improved varieties of finger millet to be grown in the area during the March and June rains (ICRISAT, 2011). Millet is dehulled and milled to produce flours, grits and dehulled whole grains. These products are used to prepare staple foods such as, thin and thick porridges, bread and steam cooked products. The grain is also used to make alcoholic beverages, non-alcoholic beverages and snacks. The husked grain of the crop has a nutty flavor and can be eaten whole after being roasted or cooked. The green, dry leaves and the straw are used as animal feed so that the whole crop is utilized (Tatham *et al.*, 1996). Despite cases of goiter reported in Mogotio, finger millet continues to be one of the favorite food crops. As such this calls for investigation of thiocyanate levels in finger millet grown in this region.
1.2 Problem statement and justification

Mogotio is a dry area and finger millet is a staple food eaten by both children and adults. The crop is known to contain thiocyanate which can lead to goiter if there is low iodine in the food. The area has higher goiter prevalence than the recommended WHO cut-off limit. The main concern is what causes it. It was therefore important to quantify the thiocyanate levels in the red and brown varieties of finger millet grown in the area to verify whether they are safe. When using finger millet, it is processed in many ways such as drying, soaking and fermentation prior to cooking. It is not known how these processes affect the levels of thiocyanate hence the need to find out. The leaves of the crop are eaten by animals which provide meat and milk which introduce thiocyanate. It was therefore important to determine the levels of thiocyanate in the leaves.

1.3 Hypotheses

i. The content of thiocyanate in the fresh dried, sprouted and soaked grains, leaves, fresh flour, fermented and cooked flour from the red and brown finger millet from Mogotio, Baringo County are not significantly different.

ii. Drying, sprouting and soaking of grain does reduce the levels of thiocyanate in the red and brown finger millet grown in Mogotio, Baringo County
1.4 Objectives

1.4.1 General objective

To determine the concentration of thiocyanate in the processed and unprocessed red and brown finger millet grown in Mogotio Baringo County.

1.4.2 Specific objectives

i. To determine concentration of thiocyanate in the fresh, dried, sprouted and soaked grains of the red and brown finger millet from Mogotio in Baringo County.

ii. To determine the concentration of thiocyanate in the fresh, fermented and cooked flour of the red and brown finger millet from Mogotio in Baringo County.

iii. To determine the concentration of thiocyanate in the green and dry leaves of the red and brown finger millet from Mogotio in Baringo County.

1.5 Significance of the study and expected output

The findings from this study provide a profile of levels of thiocyanate in the leaves and the grains and further the levels of thiocyanate as the finger millet undergo processing for consumption. The results obtained indicate the best method of processing finger millet so as to lower thiocyanate level before consumption. The documented results will be published and sent to the relevant authorities like the public health to benefit the people of Mogotio and improve the health of everybody taking finger millet. It is anticipated that consumers will adhere to the recommended form as projected in these findings.
1.6 Scope and Limitations of the study

Among the various varieties of millet including *Pennisetum, Setaria, Paspalum, Eleusine* and *Echinochoa, Eleusine* was only analysed for thiocyanate. Red and brown varieties of this finger millet were investigated, although the other varieties (white and black) do exist, though not grown in the study area. The effect of heat, fermentation, sprouting and soaking on thiocyanate content was determined. Soaking and cooking time was also varied. The other finger millet products like bread, alcoholic and non-alcoholic beverages were not investigated for thiocyanate levels. The effect of storage conditions on thiocyanate levels was not determined.
CHAPTER TWO
LITERATURE REVIEW

2.1 Goiter prevalence

Goiter is a swelling of the thyroid gland which can lead to swelling of the neck or larynx. The disease is associated with hypothyroidism or hyperthyroidism. Goiter cases are minimal in affluent countries where table salt is supplemented with iodine. However, it is still prevalent in India, China, East and Central Africa. In Africa, goiter is endemic in many countries notably Congo, Uganda, Kenya, and Sudan. The prevalence is as high as 81% in some parts of these countries (Elnour et al., 2000). It is the chief consequence of iodine deficiency, resulting from either low iodine intake or ingestion of goitrogens (Narwal, 2013).

A national micronutrient survey conducted in 45 districts in Kenya found total goiter rates as 16.3% (FAO, 2005). Three had no goiter problems, while 30 had mild iodine deficiency disorders (IDD) with goiter prevalence between 5 and 19%, 7 had moderate problem (TGR 20-29%) and 5 districts were noted to have goiter prevalence’s greater than 30%. Survey data from Rift Valley where Mogotio is situated indicated total goiter prevalence as 20% (FAO, 2005). Cases of goiter have been reported in Mogotio despite the use of iodized salt, raising a public health concern (Adwok, 2006). Furthermore, doctors who carried out goiter surgical operation services in three towns of the north Rift region including Kitale, Kapenguria, and Lodwar reported that goiter disease is highly prevalent (Wamalwa, 2012).
Goiter is conveniently referred to as endemic when it occurs in more than 10% of the population in a defined area. A TGR of 5% or more is now recommended as the cut-off point to indicate a public health problem as per the World health organization (WHO) (Adwok, 2006). This recommendation is based on the observation that goiter prevalence rates between 5% and 10% may be associated with a range of abnormalities including inadequate urinary iodine (UI) excretion or subnormal levels among adults, children and neonates. WHO recommends that iodine deficiency surveys should examine school-age children (6-12 years) because of their high physiologic vulnerability and their accessibility through school for studies in baseline health parameters (Hashemipour et al., 2007).

Iodine which is essential for the formation of thyroid hormones is found primarily in sea water and in the soil in coastal areas. In the developing world, people who live inland or at high elevations are often iodine-deficient and can develop goiter when the thyroid enlarges in an effort to obtain more iodine. The initial deficiency may be made worse by a diet high in thyroid hormone inhibiting foods such as cabbage, broccoli, cauliflower, cassava and millet (Adwok, 2006). Despite sufficient iodine intake and ingestion of unknown goitrogens, causes like protein-energy malnutrition, vitamin A, iron, selenium and zinc deficiencies or their combination may be responsible causes (Hashemipour et al., 2007). Other risk factors that may predispose one to goiter include; family history of goiter, female gender and age over 50 years. Common symptoms of goiter are weight gain, fatigue, low blood pressure, fluid retention, depression and body pain. The swollen
thyroid can put pressure on the windpipe and oesophagus, which can lead to coughing, hoarseness, swallowing and breathing difficulties (Norman, 2011).

2.2 Millet

Millets are a group of highly variable grasses with many small seeds, they belong to the *Poales* order and to the family of *Gramineae*. The most important millets are pearl millet (*Pennisetum glaucum*), finger millet (*Eleusine Coracana*), Proso millet (*Panicum Miliaceum*) and foxtail (*Setaria italic*a). Other types of regional importance include Fonio (*Digitaria exilis*), Kodo (*Paspalum scrobiculatum*) and teff (*Eragrostis tef*). *E. Coracana* is well adapted to the arid and semi-arid regions where it acts as a security food crop (Makokha, 2002). Pearl millet is the most widely grown of all millets and the highest yield potential of all millets under drought and heat stress. Foxtail millet is mostly cultivated in Europe, India, Indonesia, Korean Peninsula and the former U.S.S.R (Dendy and David, 1993).

Millets are better adapted than most crops to dry, infertile soils with poor water-holding capacity. Statistics indicate that 50% of total millet production in Kenya is pearl millet, 30% of proso/golden millet, 10% foxtail millet and 10% finger millet (FAO, 2005). The low and uncertain rainfall in Mogotio restricts farmers to cultivate drought resistant crops such as finger millet (Peter, 1992). The soils in the area are shallow, gravely and stoney derived from rocks and alluvial deposits. They also have a low water holding capacity and are easily eroded (Kubo *et al.*, 2008).
Several factors including storage conditions affect the nutritional quality of finger millet and being a staple consumed at household levels, processing must be considered at both traditional and industrial levels, involving, small, medium and large scale entrepreneurs (Obilana and Mangasa, 2002). Soaking, sprouting, cooking and fermentation of millet reduced antinutrients found in pearl millet seeds to lower levels to a greater extent than sun and oven-drying (Ikemefuna, 1994). Boiling reduced phytic acid while combination of boiling and roasting are effective in lowering the levels of cyanogenic glycosides. These processes produce enzymes that breakdown complexes to free tannins which are then leached out. Soaking and fermentation decreased the tannins content. By adequate processing both the cyanogenic glycosides and thiocyanate can be removed or reduced prior to consumption thus significantly reducing the potential health risk (FSA, 2005).

2.2.1 Finger millet

*Eleusine coracana* (Finger millet) is a highly tillering annual grass, whose average height is a little over 1.0 m, but can reach as high as 1.6 m. Tillers come from the base of the plant and axillary buds along the stem, each tiller produce a panicle. Leaves are generally 30 cm to 40 cm and are narrow (1.5 to 3 cm). Panicle branches commonly come from the same place giving a finger like appearance (Kajuna, 2001).

The colour of the grains may vary from white, orange and red, brown, purple to almost black. Finger millet known as ragi in India, can survive in areas with as little as 300 mm or less of seasonal rainfall and it can also grow well in high rainfall areas. It is cheaper to grow as compared to other crops such as maize, rice and wheat. The crop is not easily
attacked by diseases and striga weed, thus yielding more economic profits (Angwenyi, 2013). Finger millet matures in about 3-5 months time, depending on variety. Harvesting is the most labour intensive operation of the production. The ripe heads are individually picked with knife and dried in the sun at a clean hard ground. Good care is needed from any physical mixing during harvesting and drying. Mixing can easily occur during these operations. When heads are dried enough, then they can be threshed and winnowed. This way, a good quality seed can be produced. However, combine harvesters have been used in large scale production such as in Zimbabwe when the crop is fully mature. A normal seed crop may give seed yields ranging from 2000 to 3000 kg/ha. (Ocheme, 2007).

2.2.2 Uses of Finger millet

Finger millet is an indispensable food to millions of people inhabiting the semi-arid tropics where it is used primarily for human food and remains a major source of calories (Ocheme, 2007). The crop is highly nutritious especially recommended for children, convalescents and the elderly. Several food preparations are made from finger millet. They differ between countries and even between different parts. The grains are dehulled and milled in order to produce flours, grits and dehulled whole grains. These intermediate products are used to prepare staple foods like cooked grains; thin and thick porridges, preparations deep fried in oil, flat bread, mostly unleavened and prepared from fermented or unfermented dough. Millet grain is used in the production of two types of beer: clear beer and opaque beer. The latter is a traditional low alcohol African beer that contains suspended particles. It is a major ingredient in home-brewed beer. By applying
hydrothermal technologies (flaking, puffing, extrusion, micronizing) new finger millet products of good taste quality can be produced (Le'der, 2004). The husked grain has a nutty flavor and can be eaten whole after being roasted, cooked or boiled. The leaves and straws are used as animal feed, fuel and as building material, so that the whole crop is utilized (Tatham et al., 1996).

2.2.3 Antinutrients in finger millet

There is a wide distribution of biologically-active constituents throughout the plant kingdom, particularly in plants used as animal feed and in human nutrition (Soetan and Oyewole, 2008). Studies have identified certain, nutritional inhibitors and toxic substances associated with finger millet grains. Nutritional inhibitors are classified broadly, as those naturally present in the grains and those due to contamination, may be of fungal origin or may be related to soil and other environmental influences. These modify the nutritional value of the grains and some of them have very serious consequences (Ocheme, 2007). Antinutrients in finger millet include cyanide (cyanogenic glycosides), goitrogens, polyphenols, phytates, tannins, oxalic and phytic acid which are plant constituents which play an important role in biological function of the plant. The effect of these compounds on human and animal organisms is partly negative because they can reduce digestibility of nutrients and the absorption of minerals. They may also inhibit growth as a result of their negative influence on the function of pancreas and the thyroid gland and can cause pathological alteration in the liver. Cyanogenic glycosides occur in most millet varieties. Dhurrin (a cyanogenic
glycoside present in millet) is found mainly in the leaves and germinating seeds of millet can amount to 3-4% of the total dry seedling weight (Le'der, 2004).

Previous studies reported a high incidence of goiter in the millet-consuming population of Sudan, which has not been observed in the millet-consuming population of Nigeria. Varietal differences in the C-glycosyl flavanol (C-GF) content of millet, the method of processing millet into foods may affect the incidence of goiter in millet-consuming populations (Akingbala, 1991). There is less information about the goitrogenic action of millet, but Gaitan (1990) mentioned goitrogens in millet and identified certain flavonoids that interfere with iodine utilization in rats. Thiocyanate is also present in millet and its antithyroid effects are additive to those of the C-GF. Indeed, the thiocyanate content of Pennisetum millet is similar to that present in cabbage (3.5 mg/100g) a vegetable of the Brassica genus that is a reputed goitrogen (Gaitan et al., 1989). A study carried out in Guinea, West Africa confirmed that high consumption of millet and cassava was responsible for the high incidence of goiter in the region (Toure et al., 2003).

2.3 Goitrogens and cyanogenic glycosides

2.3.1 Goitrogens

Goitrogens are chemical substances that suppress thyroid function. Some goitrogens induce antibodies that cross-react with the thyroid; others interfere with thyroid peroxidase (TPO), the enzyme responsible for adding iodine during production of thyroid hormones (Dasgupta, 2008). These substances are found in foods such as cassava, millet, maize, bamboo shoots and sweet potatoes (Adwok, 2006). Their main source is the
cyanogenic glycosides and glucosinolates found in the plant kingdom. When the diet is overly rich in goitrogens, the thyroid gland swells to trap as much iodine as possible forming a goiter or a lump in the neck.

Cyanogenic glycosides are secondary metabolites present in more than 2500 plant species; they are considered to have an important role in plant defense against herbivores due to their bitter taste and release of toxic hydrogen cyanide upon tissue disruption (Zagrobelny, 2008). Cyanogenic glycosides (thiocyanate precursors) are found in several staple foods such as cassava, millet and sorghum which are foods in many countries. Thioglycosides undergo a rearrangement to form isothiocyanate derivatives and in some instances thiocyanate (Rask et al., 2002). Therefore, the amount of thiocyanate in the urine is a good indicator of the presence of thioglycosides in food. Thiocyanate or isothiocyanate like compounds primarily inhibit the iodine – concentrating mechanism of the thyroid, and their goitrogenic activity can be overcome by iodine administration (Chandra et al., 2004).

2.3.2 Biosynthesis, catabolism and detoxification of cyanogenic glycosides

The main metabolic processes resulting in synthesis, degradation and detoxification of cyanogenic glycosides (CNGs) are shown in figures 2.1, 2.2 and 2.3 respectively. These processes lead to the formation of thiocyanate. The two committed steps in CNGs biosynthesis are catalyzed by cytochromes P450, via two successive N-hydroxylations of the amino group of the parent amino acid followed by decarboxylation and dehydration. (Zagrobelny et al., 2004).
The aldoxime formed is subsequently converted to an alpha-hydroxynitrile through the action of a second cytochrome P450. This reaction involves an initial dehydration reaction that forms a nitrile and is followed by hydroxylation of the alpha carbon to generate cyanohydrins. The final step in CNG synthesis, glycosylation of the cyanohydrins moiety, is catalysed by a UDPG-glycosyltransferase (Zagrobelny et al., 2004).

**Figure 2.1** Biosynthesis of CNGs in plants (Zagrobelny et al., 2004)

Catabolism of CNGs is initiated by enzymatic hydrolysis by a beta-glucosidase to afford the corresponding alpha-hydroxynitrile, which at pH values above 6 spontaneously dissociates into a sugar, a keto compound and Hydrogen cyanide.

**Figure 2.2** Catabolism of CNGs (Ilza and Pinotti, 2000)
HCN is detoxified by two main reactions. The first route involves the formation of beta-cyanoalanine from cysteine and is catalyzed by beta-cyanoalanine-synthase. The second route proceeds by conversion of HCN into thiocyanate and is catalyzed by rhodanese (Bordo and Bork, 2002). The detoxification route involving beta-cyanoalanine is common in plants and possibly also in insects, while the thiocyanate pathway occurs mainly in vertebrates but also some plants and insects.

![Diagram of detoxification of CNGs](Zagrobelny et al., 2004)

### 2.3.3 Glucosinolate hydrolysis and products

Glucosinolates are secondary metabolites that can help plants defend themselves against phytophagous insects, fungi and other pests, they are hydrolysed by thioglucosidase (myrosinase) enzymes to yield a glycone which undergoes non-enzymatic re-arrangement to produce organic isothiocyanates, thiocyanates, nitriles and other products as shown in figure 2.4 (Tsao, 2002).
Although glucosinolates (thioglycosides) have protective qualities some have been reported to cause goiter when consumed in large quantities and for this reason should be classified as toxins (Vaughan and Geisser, 2009). They are broken by enzymes in food when it is cut or grated to form thiocyanate, isothiocyanate and thiooxazolidone which exhibit a clear antithyroid activity. Isothiocyanates can spontaneously form a ring shape molecule (goitrin) that interferes with the thyroid and is goitrogenic) (Chandra et al., 2004). The ingestion of substantial amount of glucosinolates (Gls) may be deleterious to animal health and production. Upon ingestion, the intact Gls and/or their breakdown products are absorbed from the intestinal lumen and/or converted into other products. Gls are known to induce iodine deficiency, hypertrophy of liver, kidney and thyroid and at higher levels, mortality. Deleterious effects of Gls are greater in non-ruminant animals.
compared to ruminants. Young animals are more sensitive to Gls than adult and older animals. High Gls ingestion in poultry increased mortality and lowered egg production as well as egg weights, whereas Gls are fatal to pigs. Studies with fish also revealed deleterious effects of Gls on growth inhibition and thyroid function (Tripathi and Mishra, 2007).

2.4 Thiocyanate

Thiocyanates are a group of compounds formed from a combination of sulfur, carbon, and nitrogen. Thiocyanates are found in various foods and plants. They are produced primarily from the reaction of free cyanide with sulfur. It is also a break down product of Gls. Although thiocyanates are less harmful than cyanide in humans, they are known to affect the thyroid glands, reducing the ability of the gland to produce hormones that are necessary for the normal function of the body (ATSDR, 2006).

Dietary or nutritional thiocyanate is a very important substance necessary for optimal health and well being. Thiocyanate is found in specific foods, common to the indigenous African diet as well as some Middle Eastern and Mediterranean diets. When thiocyanate is present in the diet, it acts as an oxygen carrier and increases the capacity of the blood to transport the life-giving oxygen to every single cell of the body. Because of its oxygen–enhancing properties, a diet rich in thiocyanate is effective in helping mitigate sickling of the red blood cells (Medani et al., 2010).
Thiocyanate promotes cell growth, has protective properties in case of toxic and mutagenic cell exposure and stimulates the immune response and the phagocytosis (Brauer et al., 2006). Nitrilosides and thiocyanate plant foods are known to prevent high blood pressure, arthritis and rheumatism, gastrointestinal disorders and cardiovascular disease. If the body lacks in a sufficient diet rich in nitrilosides and thiocyanate plant foods, it causes a decrease in the number of favorable intestinal bacteria and a subsequent increase in unfavorable organisms. This may lead to constipation, yeast infections, colon and rectal cancer (Kirk, 2010).

### 2.4.1 Thiocyanate content in food plants

Studies by Chandra et al. (2004) and Malik et al. (2012) reported the levels of thiocyanate in cassava, cabbage, cauliflower, mustard, rape kale, beans, sweet potatoes and carrots. Gaitan et al. (1989) reported the levels of thiocyanate in pearl millet. The levels of thiocyanate in selected food plants are shown in table 2.1. Thiocyanate content was described as high in these plants and that, in addition to iodine deficiency dietary intake of cyanogenic plant having high thiocyanate content may play some role for the persistence of endemic goiter during the post iodization period (Malik et al., 2012).
Research on thiocyanate content of kales in various kale growing areas in Kenya reported levels of thiocyanate in µg/g as: Nakuru 159, Kericho 490, Karatina 502 and Limuru 2802 (Chweya, 1990). It was concluded that thiocyanate level in plants vary from one region to another due to differences in the soils and seasonal variations. Thiocyanate content in the soil varies depending on what was initially present in the land before planting, the type of agrochemicals such as fertilizers, herbicides and pesticides used. Nitrogen fertilizer application has been reported to affect thiocyanate levels in kale leaves. Further studies confirmed that thiocyanate content in plants is high during the rainy season as compared to the dry season and the levels were high in the shoots and leaves than in the dry leaves (Chweya, 1990). A study on sorghum showed that level of thiocyanate precursors (HCN) are affected by age, genotype, temperature, phosphorus
nutrition and possibly light intensity (Wheeler and Mulcathy, 1989). Hydrogen cyanide potential of sorghum leaves is usually in the range 100-800 µg/g with few exceptions exceeding 1000 µg/g. The HCN potential of sorghum after flowering may be only 10% (10-80 µg/g) of its value when young and vegetative and because of this, farmers are encouraged to wait till maturity in order to feed their animals with sorghum (Ilza and Pinotti, 2000, Wheeler and Mulcathy, 1989).

2.4.2 Role of thiocyanate in goiter development

Thiocyanate (SCN) is a complex anion which is a potent inhibitor of iodide transport. It is the detoxification product of cyanide and can easily be measured in body fluids (Erdogan, 2003). The development of goiter is critically related to the balance between iodine and thiocyanate, a goitrogen found in some African diets (Toure et al., 2003). Thiocyanates make it harder for the gland to absorb iodine because they compete with iodine for entry into the gland. This effect can be minimized by supplementing the diet with iodine, where the excess iodine can then crowd out the thiocyanate (FAO, 2008). Consumption of naturally occurring goitrogens, certain environmental toxins and cigarette smoking can significantly increase SCN\(^{-}\) concentrations to levels capable of affecting the thyroid gland. Goiter endemics were reported to develop when the critical urinary iodine/SCN ratio decreases below 3 microgram iodine per mg SCN\(^{-}\) (Erdogan, 2003).

Studies on cows have indicated that thiocyanate may have effect on goiter development as well (Bobek, 1992). Thiocyanate belongs to goitrogenic compounds and its main source are the plants of brassica species widely cultivated (Bobek, 1992). High intakes of
cruciferous vegetables and millet have been found to cause hypothyroidism (insufficient thyroid hormone). There has been one case reported of an 88-year old woman developing severe hypothyroidism and coma following consumption of an estimated 1.0-1.5 kg/day of raw bok choy for several months (Higdon, 2005). Two mechanisms have been identified to explain this effect. The hydrolysis of some glucosinolates may yield a compound known as goitrin which has been found to interfere with thyroid synthesis. The hydrolysis of another class of glucosinolates, results in the release of thiocyanate ions, which can compete with iodine for uptake by the thyroid gland (Higdon, 2005).

Raw cabbage contains high doses of SCN\(^-\) therefore unbalanced nutrition with cabbage has been associated with increased thyroid volume and goiter. This may be relevant especially in regions with low I/SCN\(^-\) ratios caused by iodine deficiency and additional excessive consumption of cabbage and may thus contribute to the development of endemic goiter (Brauer, 2006). Further studies also confirmed that thiocyanate inhibition of iodine transport is operative in humans with a high thiocyanate intake. At low or medium intakes of thiocyanate all effects on the thyroid can be prevented by iodine supplementation, but not at high levels of intake. Various studies indicate that thiocyanate generated from glucosinolates in rapeseed inhibits NIS in the mammary gland of the lactating animal. This leads to a low iodine content of milk and thereby an impaired thyroid function in the piglet, because of iodine deficiency. The effect of thiocyanate in feeding on iodine content of milk in the lactating cow and sow has some implications for our understanding of iodine deficiency in breast fed children. If the area where the mother and child are living is characterized by iodine deficiency and the diet
of the mother is rich in thiocyanate or substances generating thiocyanate, the iodine content of the mothers' milk will be low. Hence the child may be severely thyroid hormone deficient because of lack of iodine, even if the thiocyanate excretion in milk is low (Laureberg et al., 2002). Virion and colleagues (2005) summarized the effect of SCN\(^-\) by studying two reactions: thyroglobulin iodination and thyroid hormone synthesis (coupling reaction) catalyzed by peroxidases and made the following conclusions: SCN\(^-\) inhibits iodide oxidation (I\(^-\) \(\rightarrow\) I\(_2\)) whatever the enzyme, thyroid, peroxidase or horse radish peroxidase. The amount of SCN\(^-\) required to completely inhibit this reaction varies depending on the enzyme (Virion et al., 2005). Similarly tyrosine iodination is inhibited by SCN\(^-\) with large variations, depending on the peroxidase, in the concentration of this anion for inhibition. In contrast SCN\(^-\) stimulates the coupling reaction: this effect is seen with the thyroid and lactoperoxidase; the concentration of SCN\(^-\) required for half-maximal stimulation of the coupling reaction is much lower (0.5-1 \(\mu\)M) than that required for the inhibition of iodide oxidation (60-80 \(\mu\)M) (Virion et al., 2005). The normal level of thiocyanate in blood serum should not exceed 100 mg/L (WHO, 2003).

Perchlorate (CIO\(_4^-\)), an anion with the same molecular size as SCN\(^-\) and I\(^-\), has no effect on the coupling reaction; this stimulatory effect of SCN\(^-\) does not depend on a modification of the thyroglobulin molecule since it is not seen with horse radish or in purely chemical coupling conditions. The stimulatory effect of SCN\(^-\) is therefore seen as resulting from the binding of this anion to a limited number of high affinity sites present at the surface of both thyroid and lactoperoxidases. The inhibitory effect
depends, in contrast, on the binding of SCN\(^-\) to the substrate site with lower affinities. Since iodide also behaves both as a substrate for the iodination reaction these information further support in favour of the existence of an enzyme- iodide (or SCN\(^-\)) complex with catalytic properties different from those of the native peroxidase (Virion \textit{et al.}, 2005).

2.5 Food processing and thiocyanate levels
A better understanding of the effects of different processing methods on thiocyanate levels may lead to wider use of finger millet in the food industry. The overall thiocyanate content in a plant directly correlates with the amount of the cyanogenic glycosides (cyanide) and glucosinolates that are present in the plant. Therefore factors that are effective in lowering cyanide also lead to lower thiocyanate level (Zagrobelny \textit{et al.}, 2004).

2.5.1 Heat
Studies on cassava reported that heating, ensiling and fermentation are the most effective ways of eliminating cyanogenic substances whereas oven drying method is the least effective. Sun-drying cassava peel results in a greater loss of cyanogenic glycosides 264.3-321.5 ppm compared to laboratory oven drying 666.8-1250 ppm at 60\(^\circ\)C for 48 hours (Tewe, 2003). Freshly harvested sorghum leaves contained 353 ppm HCN, leaves air dried for seven days contained 155 ppm and leaves chopped then air dried was found to contain 82 ppm HCN (Wheeler and Mulcathy, 1989). Sun-drying also tends to produce greater loss of bound cyanide due to slower rate relative to oven drying and allows a
longer contact period between the glycosidase and the glycoside in the aqueous medium.
The effectiveness of the enzyme/substrate interaction will however depend on the particle size and the environmental factors such as ambient temperature, insulation, relative humidity and wind velocity. Thus proper sun drying of finger millet is achieved between 1-3 days in the dry season and in up to 8 days during the rainy season. Furthermore sun drying facilitates the continuation of the fermentation process (Asegbeloyin and Onyimonyi, 2007). The residual level of thiocyanate in processed food would therefore depend on the processing method (Kittivachra, 2006). Cooking destroys active enzymes involved in thiocyanate formation at about 72°C leaving a considerable portion of glycoside intact (Tewe, 2003). Heat treatment negatively affects glucosinolates content, wet heating/pressure cooking is more effective over dry heating (Jensen et al., 2001). Microwaving reduces the average thiocyanate yield to one half; steaming reduces this yield to one-third. The effect of microwaving and steaming is dependent on the individual’s intestinal flora and is thus highly variable, whereas the effect of boiling is more reliable and constant (Master, 2008).

The observation that autoclaving of millet reduced its goitrogenic properties supported the volatile or heat labile nature of the active principle. Studies have also confirmed that combination of drying and cooking reduced levels of thiocyanate than cooking alone (Tewe, 2003). The Glucosinolates degradation takes place at each level of feed processing starting from oil extraction to diet preparations. The heating reduces glucosinolates depending on the type of compound, degree and time of heating (Tripathi and Mishra, 2007). Thiocyanate in plants foods could be bound, free or volatile. Each of
these forms of thiocyanate respond differently to processing. Women with thyroid problems should not therefore avoid cruciferous vegetables or millet but steam or cook, as heat alters the isothiocyanates molecular structure and eliminates goitrogenic effect (Marcelle, 2011).

A study on detoxification of cassava leaves revealed that pounding cassava leaves in a mortar and allowing to stand for 2 hours and cooking with coconut scrapings led to reduction of total and free cyanide to <0.5 mg/kg fresh weight of cassava leaf (Priyadarshani et al., 2004). The experiment shows that pounding and allowing to stand for 2 hours activated a very active linamarase. A similar lowering was observed on boiling water treatment involving addition of limited amounts of boiling water to the cassava leaves before cooking. The study also observed that cooking cassava leaf in the traditional method yielded unacceptable levels of cyanide (110-120 mg/kg fresh weight), which can lead to chronic cassava toxicity in general including goiter. This was found to be the clear cause for the high prevalence of goiter in the arid areas of Monaragala, Sri-lanka (Priyadarshani et al., 2004).

2.5.2 Fermentation

The importance of fermentation is based on its ability to reduce the cyanogenic glycosides to insignificant levels. A case study on the effect of processing methods on the residual cyanide of cassava flour, fermentation proved efficient in reducing the total cyanide content from levels as high as 224.09±0.858 ppm to 86.63±1.049 ppm after 2 days (Asegbeloyin and Onyimonyi, 2007). Furthermore it was found that microbial
fermentation gradually decreases the glucosinolates levels to zero (Tripathi and mishra, 2007). The relatively high thiocyanate content in unfermented plant products may result from the high percentage of bond thiocyanate before being cooked. On the other hand, the fermented flour which had enough time had the opportunity for their bond thiocyanate to be hydrolyzed and thus distributed to different forms and the volatile HCN content removed during cooking. Fermentation inactivated the enzyme myrosinase thus reduced the total glucosinolates content. The biochemistry and microbiology of the fermentation process is only superficially understood but it is believed that some cyanidrophilic tolerant micro-organisms effect the breakdown of the cyanogenic glycosides (Tewe, 2003). This reduction may be due to utilization of glucose and sulphur moieties of the compounds by microbial enzymes (Vig and Walia, 2001).

2.5.3 Sprouting

Steeping of grains cereals in water followed by germination or sprouting is a common household practice in developing countries including Kenya (Chove and Mamiro, 2010). Sprouting reduces the high viscosity and water-binding characteristic of starch based porridges. As soon as the cereal and legume seeds are hydrated, chemical changes occur, which result in partial breakdown of storage components, such as starch and proteins. The starch nature of the un-sprouted grains allows these foods to bind so much water yielding a thick porridge (Onyeka and Dibia, 2002). Dilution of the porridge increases bulk and renders the food more difficult for infants to consume in one sitting. The bulkiness of the porridge limits adequate amount of nutrient intake by the infants.
Thus sprouting is mainly used to lower dietary bulk in cereals because it converts significant amounts of starch, which is principally responsible for viscosity in cereal gruels, to sugars and short chain oligosaccharides (Traoré et al., 2004). In the process of sprouting of seeds, cyanides are equally mobilized not only to take part in some metabolic processes but also as a protective or defense mechanism agents when the shoot is emerging (Chove and Mamiro, 2010).

Sprouting of millet seeds results in increase in thiocyanate levels. Research carried out on two nigerian cultivars of finger millet Maiwa and Gero reported that sprouting (germination) increased the cyanide content by 2.11 to 2.14 fold in the two varieties (Salami, 1994). The cyanide content increased linearly as the number of days of sprouting of the millet grain increased and the highest values of cyanide were attained on the third day of sprouting. The grains of the same cultivars were sprouted for periods up to 96 hours. Maiwa had a higher cyanide content 1.82±0.1 mg/kg than Gero 1.33±0.1 mg/kg relative to the weight of ungerminated dry millet grains (Salami, 1994). The seeds of four cultivars of grain sorghum and four of sweet sorghum (sorghum bicolor) contained only traces (1 or 2 ppm) to 29 ppm of potential hydrocyanic acid (HCN) that could be generated by digestion and steam distillation. Sprouts of the same cultivars grown for 3 days however contained 258-1030 ppm potential HCN relative to the weight of the ungerminated dry seed (Oksana and Bills, 2006). Drying at 50°C and grinding of sprouts to produce a meal did not reduce the potential HCN content. The consumption of sorghum sprouts or products may therefore be hazardous (Oksana and Bills, 2006).
Studies on young bamboo shoots or sprouts fed on rats for a period 45 to 90 days found that the rats had a significant increase in the thyroid weight. The rats also had higher excretion of thiocyanate and iodine with marked decrease in thyroid peroxidase activity (Tripathy, 2004). The high levels of thiocyanate in the young shoots or sprouts could be attributed to the enzymes which are active during the young growing stages of plants (Chweya, 1990). Previous studies reveal that the problem of production of cyanogenic glycoside dhurrin from sorghum during sprouting is manageable. Firstly, the glycoside and the dhurrin-synthesising enzyme are primarily located in the coleoptile, in a young shoot. Other workers have reported quite low levels in roots and seeds. This implies that removal of the shoots after sprouting may help to minimize this problem (Chove and Mamiro, 2010). Further studies revealed that thiocyanate in the shoots and leaves increased during the rainy season than in the dry season (Chandra et al., 2004).

2.5.4 Soaking

The residual level of thiocyanate in any food depends on the amount of cyanogenic glycosides remaining after processing. A study on cassava found that soaking provides a suitably larger medium for fermentation and allows for greater extraction of the soluble cyanide into the soaking water (Adamafio and Ankrah, 2009). The process removes about 20% of the free cyanide after 4 hours although bound cyanide is only negligibly reduced. Bound cyanide begins to decrease only after onset of fermentation. A very significant reduction in total cyanide is achieved if the soaking water is routinely changed (Tewe, 2003). Soaking in water improves detoxification as cells are broken by osmosis and fermentation which facilitates hydrolysis of the glycosides. Longer soaking periods (18 to
24 hours) can reduce cyanide levels by 50%. For example cassava roots soaked for 3 days led to reduction of cyanide from 25.5 to 19.4 mg/kg (FAO, 2008). Cassavas soaked in water for 24 hours followed by sun-drying for 24 hours resulted in 63-74 percent reduction in cyanogenic glycoside levels compared with 27-64 percent reduction after sun-drying for 48 hours (Adamafio and Ankrah, 2009).

2.6 Method of analysis of thiocyanate

Different methods can be used to determine thiocyanate in food including fluorometry, spectrophotometry, chromatography, picrate method and electroanalytic approaches. These methods have a short linear range, time consuming and expensive (Vanfard et al., 2012). The picrate method is insensitive or of qualitative interest only. The ferric thiocyanate procedure although of reasonable sensitivity is extremely unreliable with small amounts of thiocyanate owing largely to instability of the ferric thiocyanate colour. In the present study acid hydrolysis (method of Aldridge) with UV-VIS spectrophotometric detection was used for determination of thiocyanate. The method is accurate and can measure trace quantities of thiocyanate (Chandra et al., 2004)

2.6.1 Principle of acid hydrolysis

The method is based on conversion of thiocyanate by bromine water into cyanogen bromide. The reaction is shown in equation 2.1.

\[
\text{KCNS + Br}_2 + 4\text{H}_2\text{O} \rightarrow \text{KBr + CNBR + H}_2\text{SO}_4 + \text{HBr}
\]

…………….. Eq. (2.1)
The cyanogens bromide thus formed after removal of excess bromine using arsenous trioxide, reacts with a solution of phenyl-diammine in dilute pyridine to give an intense orange to red-colour proportional to the amount of cyanogens bromide present. By this means 0.6µg of thiocyanic acid may be easily and accurately estimated using UV-VIS spectrophotometer. The limit of detection on a rough qualitative basis is of the order 0.05µg of thiocyanate (Malik et al., 2012).

2.6.2 UV-VIS spectroscopy

Spectrophotometers are spectrometers that allow measurement of the ratio of two beams, a requirement to measure absorbance. It measures the intensity of light passing through a sample (I), and compares it to the intensity of light before it passes through the sample (I₀). The ratio is called the transmittance. The absorbance, A, is based on the transmittance (T):

\[ A = \frac{-\log (%T)}{100} \]  
\[ \text{(Eq 2.2)} \]

Samples for UV-VIS spectrophotometry are most often liquids, although the absorbance of gases and even solids can be measured. Samples are typically placed in a transparent cell, known as cuvette, this allow radiation to pass over the special region of interest. The most widely applicable cuvettes are made of high quality fused silica or quartz because they are transparent throughout the UV-Visible and near infrared regions. For a fixed path length, UV/VIS spectroscopy can be used to determine the concentration of the absorber in a solution following the principle of the Beer-Lambert law. The law states that the absorbance of a solution is directly proportional to the concentration of the absorbing species in the solution and the path length.
A = \log_{10}(I_0/I) = \varepsilon cl. ................................................................. Eq. (2.3)

Where

A - Measured absorbance in Absorbance Units (Au),

I_0 - Intensity of the incident light at a given wave length

l - Path length through the sample (in cm)

c - Concentration of the absorbing species

\varepsilon - a constant as the molar absorptivity or extinction coefficient (Skoog et al., 2007).

Spectrophotometers offer the considerable advantage that the wavelength can be varied thus making it possible to record absorption spectra. The basic parts of a spectrophotometer are a light source, a holder for the sample, a diffraction grating in a monochromator or a prism to separate the different wavelengths of light and a detector. It is a widely used technique for quantitative analysis, used as an adjunct to other spectrometric techniques in the structural analysis of organic materials and its relative precision is 0.5-5% (Fifield and Kealey, 2000). A spectrophotometer can be either single or double beam, in a double beam instrument; the light is split into two beams before it reaches the sample one beam is used as the reference; the other beam passes through the sample.

a) Single-beam instruments

The design is that of a simple and inexpensive spectrophotometer, the spectronic 20, which is designed for use in the visible region of the spectrum. The spectronic 20 reads out in the transmittance or in the absorbance on a light-emitting diode display (LED). The
instrument is equipped with an occluder, which is a vane that automatically falls between the beam and the detector whenever the cylindrical cell is removed from its holder. The light-control device is a v-shaped aperture that is moved in and out of the amount of light reaching the exit slit. To obtain a percent transmittance reading, the digital readout is first zeroed with the sample compartment empty so that the occluder blocks the beam and no radiation reaches the detector. This process is called the 0% T calibration, or adjustment.

During analysis a cell containing the blank (often the solvents) is inserted into the cell holder, and the pointer is brought to the 100% T mark by adjusting the position of the light–aperture and thus the amount of light reaching the detector. This adjustment is called 100% T calibration or adjustment. Finally, the sample containing thiocyanate is placed in the cell compartment and the percent transmittance or the absorbance at 525 nm is read directly from the LED read out. The spectral range of the spectronic 20 is 340 to 950 nm. Other specifications include a spectral band-pass of 20nm, a wavelength accuracy of ± 2.5 and a photometric accuracy of ±2% T. The instrument may be interfaced to a computer for data storage and analysis if this option is available. Single beam instruments of the type described are well suited for quantitative absorption measurements at a single wavelength. Here, simplicity of instrumentation, low cost and ease of maintenance offer distinct advantages.

b) Double beam instruments

In this instruments two beams in space are formed by a V-shaped mirror called a beam splitter, one beam passes through the reference solution to a photo detector, and the
second simultaneously passes through the sample to a second matched photo detector. The outputs are amplified and their ratio or log of their ratio is obtained electronically or computed and displayed on the output device. Double beam instruments offer the advantage that they compensate for all but the most short-term fluctuations in the radiant output of the source. They also compensate for wide variations of source intensity with wavelength. Furthermore, the double beam design is well suited for continuous recording of absorption spectra (Skoog et al., 2004). In the present study single beam instrument was used.
CHAPTER THREE

METHODOLOGY

3.1 Research design

The experimental design involved sampling of the red and brown finger millet, sample processing which included drying, sprouting, soaking, cooking and fermentation and analysis of thiocyanate levels.

3.2 Sample collection

Random sampling was used to select the farmers in Mogotio. Sampling of the Red and brown finger millet grains was done in 2012 and 2013 between the months of October and December during harvesting time. Sampling of the grains was done two times every month during the three months. About 4.0 kg each of the red and brown finger millet grains was sampled. Two months old green leaves were also collected from the same farmers between June and August 2013. The samples were put in different plastic bags which were labeled well and taken to Kenyatta University, Chemistry laboratory.

3.3 Chemicals and Reagents

Analytical grade chemicals were used in the analysis. The chemicals included potassium thiocyanate (KSCN), de-ionized water, HNO₃ (65 % w/v), Trichloroacetic acid, saturated bromine water, Arsenous trioxide, pyridine, benzidine/phenyl diammine and hydrochloride.
3.4 Cleaning of apparatus

All glassware were soaked overnight in 10% analytical grade nitric acid, washed with detergent and rinsed with de-ionized water before being dried in an oven at 105 °C. Plastic bottles were thoroughly washed with detergent and also rinsed with de-ionized water then dried in open racks.

3.5 Preparation of the standard solution

Thiocyanate stock solution was prepared by dissolving 1.68 g potassium thiocyanate in 100 ml distilled water and then diluted to 1 litre to give 1000 µg/ml (1000 ppm) thiocyanate. The stock solution was stored in plastic bottles and was labeled appropriately. Working standards (1-15 ppm) were freshly prepared each time an analysis was carried out.

3.6 Sample preparation

Finger millet grains were cleaned by winnowing to remove dust and other extraneous materials. Unviable and broken grains were handpicked. The grains were then divided into three portions and treated as given below.

3.6.1 Preparation of the fresh dried grains

The cleaned red and brown finger millet grains were spread on clean trays and sun dried for 12 hours (Mbithi et al., 2002).
3.6.2 Sprouting of grains

Hundred grams of washed finger millet grains were put in large petri-dishes, which were covered on top with perforated aluminium foil. Sprouting was followed at intervals of 12 hours to 72 hours at 30°C. After sprouting the grains were sun dried and milled along with the rootlets to fine particles and packaged in labelled polyethylene bags awaiting thiocyanate extraction (Mbithi et al., 2002).

3.6.3 Soaking of grains

About hundred grams each of the red and brown finger millet grains were soaked in distilled water (1:2 w/v, grains to water) for 900 minutes. The water was drained and the grains transferred to large petri-dishes then sun dried for 6 hours. The procedure was repeated and the grains soaked for 10 minutes, 30 minutes and 60 minutes respectively (Chove and Mamiro, 2010).

3.6.4 Preparation of fresh flour

The dried grains of the red and brown finger millet were ground into fine flour then packaged in polyethylene weights of 100 g and stored at room temperature awaiting extraction.

3.6.5 Fermentation of flour

Hundred grams of flour prepared in section 3.6.4 was mixed with 200 ml of water and then kneaded to form dough. It was then left to ferment for 3 days at room temperature prior to thiocyanate extraction (Asegbeloyin and Onyimonyi, 2007).
3.6.6 Cooked flour (porridge) preparation

Hundred grams of the fresh flour prepared in 3.6.4 was placed in a 500 ml beaker and 200 ml of water added to make slurry. The mixture was stirred continuously with a glass rod until it boiled for 5 minutes. The cooked mixture was immediately cooled in an ice bath for 30 minutes then kept in a tightly closed plastic container awaiting extraction of thiocyanate. The procedure was repeated but the porridge was allowed to boil for 10 minutes and 30 minutes respectively (Adamafio and Ankrah, 2009).

3.6.7 Preparation of the green and dry leaves

The green leaves of the red and brown finger millet were cleaned with distilled water. The green leaves were put in a clean mortar then crushed with a pestle to give an extract that was used in section 3.7. The dry leaves were prepared by air-drying the green leaves then milled to form a fine powder.

3.7 Extraction and determination of thiocyanate

Two grams each of the red and brown finger millet samples (grains, flour and leaves) prepared in sections 3.6.1 to 3.6.7 was weighed and transferred into a distillation flask, to which 20 ml of distilled water was added. Trichloroacetic acid solution (20% w/v) was added to digest and precipitate proteins. The samples were then refluxed subsequently at room temperature for 2 hours. The dispersal of the tissue was achieved by vigorous shaking then centrifuged for 10 minutes at 1000 rpm. The supernatant containing thiocyanate was decanted into 50 ml clean dry volumetric flask and then diluted to the
mark with distilled water. It was then transferred into separate plastic bottles, labeled and appropriately stored until analysis (Chandra et al., 2004).

The sample extracts prepared were analysed for thiocyanate following the method of Aldridge (Chandra et al., 2004). Saturated bromine water and 4% arsenous trioxide was added to the extract followed by pure redistilled pyridine. 2% phenyl-diamine hydrochloride solution was added and 30 minutes was allowed for colour development at room temperature. The absorbance of the sample was read at a wavelength of 525 nm on the UV-VIS spectrophotometer (Cecil CE- 2041, 2000 series) (Chandra et al., 2004). The concentration of thiocyanate in the finger millet in µg/ml was directly interpolated from the thiocyanate calibration curve.

3.8 Method validation

To determine the accuracy of the procedure a recovery test was conducted. The recovery test was investigated by spiking a known amount of thiocyanate into a test portion of the sample and analyzing the spiked test portion along with the original sample.

3.9 Data analysis

Data was analysed using ANOVA test to compare the concentration of thiocyanate in the various forms of the red and brown varieties of E.coracana subjected to different treatments. Independent t-test was used to compare the mean values between the red and brown finger millet. Separation of means was by SNK test. Whenever a significant difference exists the means were compared at p=0.05 significance level.
CHAPTER FOUR
RESULTS AND DISCUSSION

4.1 Introduction
Levels of thiocyanate in the fresh dried, sprouted and soaked grains, fresh, fermented and cooked flour, green and dry leaves of the red and brown finger millet were determined in triplicates using a UV-VIS spectrophotometer.

4.2 Method validation
4.2.0 Regression analysis
Regression analysis was used to evaluate the linearity of the established calibration curve. The absorbance readings and concentration of the standard was used to calculate the correlation coefficient (r). The calibration curve was established by a plot of absorbance readings (y axis) against the corresponding concentration (x-axis) of the standard.

![Standard calibration curve for SCN⁻](image)

**Figure 4.1 Calibration curve of SCN⁻**
R² value from the established calibration curve y=0.018x-0.001 was 0.9992 (Figure 4.1), which shows that there was a very good correlation between concentration and absorbance.

4.2.1 Recovery test

The percentage recovery was calculated using equation 4.1 (EURACHEM guide, 1998).

\[
\text{% Recovery} = \frac{\text{SSR} - \text{USR}}{\text{USR}} \times 100
\]

\[
\text{Eq.4.1}
\]

Where

SSR− Spiked sample result
USR− Unspiked sample result

The percentage recoveries from the spiked sample (Table 4.1) ranged between 90 – 99.80%, while RSD (3.65-5.01%) which was within the acceptable range for thiocyanate (Cardoso et al., 2004). This confirms that the method is of good precision and fit for analysis of the above parameter.

<table>
<thead>
<tr>
<th>Test sample</th>
<th>% recovery</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh dried grain</td>
<td>98.91</td>
<td>4.02</td>
</tr>
<tr>
<td>Sprouted grain</td>
<td>92.97</td>
<td>5.01</td>
</tr>
<tr>
<td>Fresh flour</td>
<td>99.80</td>
<td>3.65</td>
</tr>
<tr>
<td>Cooked flour</td>
<td>95.65</td>
<td>4.21</td>
</tr>
<tr>
<td>Green leaves</td>
<td>90.01</td>
<td>5.00</td>
</tr>
</tbody>
</table>
4.3 Levels of thiocyanate in finger millet grains

The levels of thiocyanate analysed using UV-Vis spectrophotometer are presented and discussed in the following sub sections. The levels of thiocyanate in the fresh dried, sprouted and soaked grains of the red and brown finger millet are presented in table 4.2 and table 4.3.

Table 4.2 Mean levels of thiocyanate (mg/kg) in the treated grains

<table>
<thead>
<tr>
<th>Variety/Treatment</th>
<th>Concentration in (mg/kg)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Red Mean±SE (Range)</td>
<td>Brown Mean±SE (Range)</td>
</tr>
<tr>
<td>Fresh dried</td>
<td>43.48±1.56b (39.11-47.85)</td>
<td>31.83±1.88b (26.57-37.09)</td>
</tr>
<tr>
<td>n=8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sprouted</td>
<td>39.93±0.89b (37.44-42.42)</td>
<td>53.30±0.78a (51.12-55.48)</td>
</tr>
<tr>
<td>n=8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soaked</td>
<td>10.5±1.73a (2.02-18.98)</td>
<td>9.73±1.72a (1.31-18.15)</td>
</tr>
<tr>
<td>n=24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-value</td>
<td>&lt;0.001</td>
<td>0.015</td>
</tr>
</tbody>
</table>

Mean values followed by the same small letter(s) within the same column or same row are not significantly different (α=0.05, SNK-test). a<b<c

From table 4.2, thiocyanate levels in the grains of the red and brown finger millet were within safe levels (100 mg/kg). The levels of thiocyanate ranged from 43.48±1.56 to 10.5±1.56 mg/kg in the red finger millet with fresh dried finger millet having the highest and soaked having the lowest, while for brown finger millet the levels ranged from 53.30±0.78 to 9.73±1.72 mg/kg. There was a statistical significant difference between the
soaked and fresh dried grains and between soaked and sprouted in red finger millet and there was a significant difference between the fresh dried, sprouted and soaked grains in the brown finger millet. Sprouted treatment showed a significant difference between the brown variety and the red variety (P<0.001 at 95% confidence level). This could be attributed to varietal differences such as the bran and endosperm composition of the grains and metabolic breakdown of thiocyanate in the plant. The sprouted brown grains recorded the highest levels of thiocyanate followed by the fresh dried grains and the soaked grains had the least amount. The increase of thiocyanate during sprouting could have been brought about by the enzymes which are active in the shoots during the young growing stages of the plant (Chweya, 1990). The levels of thiocyanate in the fresh dried grains could be due to the fact that thiocyanate in millet is contained in the brand and endosperm portions of the seeds (Klopfenstein et al., 2012).

The levels of thiocyanate in the fresh dried and in the sprouted grains were higher as compared to the levels present in other foods such as cassava (12.95 mg/kg), cabbages (23 mg/kg) (Chandra et al., 2004) and pearl millet (35 mg/kg) (Gaitan et al., 1989). Therefore, frequent consumption of fresh dried and sprouted finger millet grains could lead to accumulation of thiocyanate in the body. Previous studies reveal that the problem of production of cyanogenic glycoside (dhurrin) from sorghum during sprouting is manageable. Secondly, the glycoside and the dhurrin-synthesising enzyme are primarily located in the coleoptile, in a young shoot. This implies that removal of the shoots after germination may help to minimize thiocyanate (Chove and Mamiro, 2010). Table 4.3 shows the effect of varying soaking time on thiocyanate content.
Table 4.3 Mean Levels of thiocyanate (mg/kg) in finger millet grains soaked at different times

<table>
<thead>
<tr>
<th>soaking time (minutes)</th>
<th>Concentration in (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Red (Mean±SE) (Range) n=8</td>
</tr>
<tr>
<td>0</td>
<td>26.35±0.45c</td>
</tr>
<tr>
<td>30</td>
<td>18.45±0.63b (13.2-18.98)</td>
</tr>
<tr>
<td>60</td>
<td>8.24±0.32a (5.23-9.6)</td>
</tr>
<tr>
<td>900</td>
<td>4.81±0.99a (2.02-7.6)</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

In Table 4.3 mean values followed by the same small letter(s) within the same column or row are not significantly different (α=0.05, SNK-test). a<b<c

Table 4.3 indicates that soaking reduced thiocyanate level in red finger millet from 26.45±0.45 to 4.81±0.99 mg/kg for 900 minutes of soaking and from 22.31±0.34 to 5.10±1.34 mg/kg for brown finger millet for the same soaking duration. There was a reduction in thiocyanate after an hour of soaking from 26.45±0.45 to 8.24±0.32 mg/kg in the red variety and from 22.31±0.34 to 8.49±0.41 mg/kg for brown finger millet after the same period of soaking. Varying soaking time significantly reduced the levels of thiocyanate (p<0.001), with longer soaking time (900 minutes) reducing thiocyanate content to low levels, 4.81±0.99 and 5.10±1.34 mg/kg for the red and brown variety respectively. There was no significant difference between soaking for 60 and 900 minutes in both the varieties. This reduction showed that thiocyanate is soluble in water and is leached away when draining water (Soetan and Oyewole, 2009). Soaking in water improves detoxification as cells are broken by osmosis and fermentation which facilitates
hydrolysis of the glycosides. Longer soaking times (18 to 24 hours) can reduce cyanide levels by up to 50%. For example, a study on cassava roots soaked for 3 days led to reduction of cyanide from 25.5 to 19.4 mg/kg (FAO, 2008). Processing of millet indicated that combination of drying and soaking was more effective in reducing thiocyanate levels than drying alone.

4.4 Levels of thiocyanate in finger millet flour

Levels of thiocyanate in the fresh, fermented and cooked flour of the red and brown finger millet are indicated in table 4.4.

Table 4.4 Mean Levels of thiocyanate (mg/kg) in treated finger millet flour

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration in (mg/kg)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Red Mean±SE (Range) n=24</td>
<td>Brown Mean±SE (Range) n=24</td>
</tr>
<tr>
<td>Fresh flour</td>
<td>20.54±1.39b (13.73-27.35)</td>
<td>24.50±1.83b (15.53-33.47)</td>
</tr>
<tr>
<td>Fermented flour</td>
<td>20.03±0.87b (15.77-24.29)</td>
<td>19.43±1.37b (12.72-26.14)</td>
</tr>
<tr>
<td>Cooked flour</td>
<td>4.28±0.50a (1.23-9.89)</td>
<td>4.96±0.40a (1.79-10.21)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Mean values followed by the same small letter(s) within the same column or row are not significantly different (α=0.05, SNK-test).
Table 4.4 indicates that thiocyanate in the fresh, fermented and cooked flour of the red and brown finger millet were within safe levels. The levels in fresh flour ranged from 20.54±1.39 in the red variety to 24.50±1.83 mg/kg in the brown variety. In fermented flour the levels ranged from 19.43±1.37 in the brown variety to 20.03±0.87 mg/kg in the red variety. Levels in cooked flour ranged from 4.28±0.50 to 4.96±0.40 mg/kg in the red and brown varieties respectively. Cooked flour had the lowest thiocyanate level, followed by fermented then fresh flour in both the varieties. Independent t-test showed that there was no significant difference between the varieties (P>0.05 at 95% confidence level). This means that consuming any of the two varieties results in absorbing relatively same amount of thiocyanate. Levels of thiocyanate in the fresh flour differed significantly from the levels present in the cooked flour (P<0.001). There was a significant difference between the levels of thiocyanate in fermented flour and in the cooked flour. Levels of thiocyanate in the fresh flour did not differ significantly from the levels in the fermented flour.

The thiocyanate content was slightly reduced during fermentation though not significant. This was due to the fact that fermentation inactivated the enzyme myrosinase thus reducing the total thiocyanate content plus also the utilization of glucose and sulphur moieties of the compounds by microbial enzymes (Vig and Walia, 2001). It is believed that some cyanidrophilic tolerant micro-organisms affect the breakdown of the cyanogenic glycosides (Tewe, 2003). Fermented flour had enough time for their bond thiocyanate to be hydrolyzed by the enzymes and thus distributed to different forms (Asegbeloyin and Onyimonyi, 2007).
Cooking caused greater reduction thus appeared to be the most effective method of reducing thiocyanate content. This was partly due to the heat sensitive nature of the active principle and the fact that cooking destroys active enzymes involved in thiocyanate formation at about 72°C (Tewe, 2003). This can also be attributed to the prior processing steps such as drying and grinding. Previous studies revealed that drying and cooking, soaking and cooking reduced levels of thiocyanate than cooking alone (Tewe, 2003). Heat treatment negatively affects glucosinolates content, wet heating/pressure cooking is more effective over dry heating (Jensen et al., 2001). Earlier studies revealed that microwaving reduces the average thiocyanate yield to one half; steaming reduces this yield to one-third. The effect of microwaving and steaming is dependent on the individual’s intestinal flora and is thus highly variable, whereas the effect of boiling is more reliable and constant (Master, 2008). Levels of thiocyanate obtained when cooking time was varied are presented in table 4.5.
Table 4.5 Mean levels of thiocyanate (mg/kg) in flour, Cooked for 5, 10 and 30 minutes

<table>
<thead>
<tr>
<th>Variety</th>
<th>Levels before cooking</th>
<th>Concentration in mg/kg</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5mins</td>
<td>10 mins</td>
</tr>
<tr>
<td>Red</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean±SE (Range)</td>
<td>n=8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20.54±1.39&lt;sup&gt;d&lt;/sup&gt;</td>
<td>8.78±0.40&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.51±0.20&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(13.73-27.35)</td>
<td>(6.58-9.89)</td>
<td>(1.89-3.30)</td>
</tr>
<tr>
<td>Brown</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean±SE (Range)</td>
<td>n=8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>24.50±1.83&lt;sup&gt;d&lt;/sup&gt;</td>
<td>8.92±0.37&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.10±0.26&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(15.53-33.47)</td>
<td>(6.85-10.21)</td>
<td>(2.9-4.9)</td>
</tr>
</tbody>
</table>

Mean values followed by the same small letter(s) within the same column or row are not significantly different (α=0.05, SNK-test). a<b<c<d

From table 4.5, the levels of thiocyanate in cooked flour ranged from 8.78±0.40 to 1.56±0.74 mg/kg in the red variety when cooking time was varied from 5 to 30 minutes, while the levels in the brown variety ranged from 8.92±0.37 to 1.85±0.63 mg/kg for the same cooking duration. There was a significant difference in the levels of thiocyanate when cooking time was varied from 5 minutes, to 10 and 30 minutes respectively (P<0.001). Cooking for 30 minutes lowered thiocyanate to very low levels implying that longer cooking time could be a sure way of reducing the levels of thiocyanate. Cooking for a short time will need inclusion of a prior treatment like soaking or sun-drying of the grains. Hydrolysis of cyanogenic glycosides yields hydrogencyanide which was driven off during boiling. Free thiocyanate was rapidly lost in boiling water (Tewe 2003; Adamafio and Ankrah 2009).
4.5 Levels of thiocyanate in the green and dry leaves of finger millet

Levels of thiocyanate in the green and dry leaves from the red and brown finger millet are presented in table 4.6.

**Table 4.6 Mean levels of thiocyanate (mg/kg) in the green and dry leaves of the red and brown finger millet**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Red</th>
<th>Brown</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SE (Range) n=8</td>
<td>Mean±SE (Range) n=8</td>
</tr>
<tr>
<td>Green leaves</td>
<td>30.78±0.40&lt;sup&gt;b&lt;/sup&gt; (28.88-32.56)</td>
<td>31.69±0.71&lt;sup&gt;b&lt;/sup&gt; (28.80-34.05)</td>
</tr>
<tr>
<td>Dried leaves</td>
<td>9.00±0.13&lt;sup&gt;a&lt;/sup&gt; (8.35-9.4)</td>
<td>8.80±0.14&lt;sup&gt;a&lt;/sup&gt; (8.25-9.15)</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Mean values followed by the same small letter(s) within the same column or row are not significantly different (α=0.05, SNK-test).

From table 4.6, mean levels of thiocyanate in the green leaves ranged from 30.78±0.40 for the red variety to 31.69±0.71 mg/kg for the brown variety. Levels in the dried leaves ranged from 9.00±0.13 in the red variety to 8.80±0.14 mg/kg in the brown variety. There was a significant difference in the thiocyanate content of the green and dry leaves of the red and brown finger millet (P<0.001). The Green leaves had the highest content of thiocyanate in both the varieties while the dried leaves had the lowest. It is therefore advisable that farmers feed their animals with the dry leaves of finger millet which contain lower thiocyanate content and not the green leaves. The high thiocyanate content in green leaves could be attributed to the enzymes active in the growing stages of plants which become inactivated during drying. It has also been revealed that environmental
conditions and agronomic factors such as plant density and nitrogen fertilizer application affect the thiocyanate levels in kale leaves (Chweya, 1990). Previous studies on cyanide potential of sorghum confirmed that after flowering HCN may be only 10% of its value when young and vegetative and thus farmers are encouraged to wait till maturity in order to feed their animals with sorghum (Ilza and Pinotti, 2000, Wheeler and Mulcathy, 1989).
CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The following conclusions were made from this study:

All the forms of finger millet analysed were found to contain thiocyanate within safe levels. The fresh dried and sprouted grain samples of the red and brown finger millet recorded thiocyanate mean levels which were significantly higher than those recorded by the soaked grains. Sprouting of brown finger millet significantly increased the thiocyanate level, therefore individuals who must use sprouted finger millet must ensure that the grains are first soaked, properly dried before fermentation and cooking is carried out to lower the thiocyanate content. Soaking greatly lowered the thiocyanate content in finger millet with 900 minutes reducing to relatively low amounts.

Cooking was found to be an effective method of lowering the content of thiocyanate to levels suitable for regular consumption especially when the cooking time was extended for 30 minutes. The levels of thiocyanate were higher in the green leaves than in the dry leaves thus farmers should feed their livestock with the dry leaves.

5.2 Recommendation

5.2.1 Recommendations from this study

i. Since finger millet has more positive health effects, people should be sensitized on the importance of processing it to lower the thiocyanate content. Processing by
soaking before cooking lowers the thiocyanate content to lower levels, which enhance regular consumption.

ii. The amounts measured in the current research would indicate that the thiocyanate contents found in the finger millet samples were below the fatal limits (100 mg/kg), but the frequency of ingestion may still result into health risks. Any procedure that reduces the thiocyanate content will thus be highly recommended in the preparation of millet foods.

iii. It is recommended that farmers should allow leaves of finger millet to dry before feeding them to animals as drying reduces levels of thiocyanate.

5.2.2 Recommendations for further study

i. Determination of thiocyanate and iodine content of other plants consumed in Mogotio should be carried out.

ii. Determination of iodine content in finger millet and in the soil where the plant is grown should be done.

iii. Determination of the iodine/thiocyanate ratio of the daily dietary intake, similarly the urinary I/SCN ratio of populations consuming millet should be investigated.

iv. Determination of thiocyanate and iodine levels in blood of those with goiter should be carried out.
REFERENCES


a) Women farmers check out finger millet at PVS trials in Baringo County

b) Farmers judge the most promising finger millet varieties in Mogotio
c) Red and brown dry finger millet grains

d) Sprouted finger millet grains
e) Cooked finger millet served with fruits
f) A map of Mogotio