VARIATION OF THIOCYANATE LEVELS IN PROCESSED RED AND BROWN VARIETIES OF FINGER MILLET (*ELEUSINE CORACANA*)

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Abstract

Finger millet (*Eleusine coracana*) is an important African staple food crop contains Cyanogenic glycosides which can be readily converted to thiocyanate by enzymes present in the plant or in the animal tissues. Thiocyanate inhibits the uptake of iodine which suppresses thyroid function leading to goiter, a disease that has documented risks. In Kenya, goiter prevalence has been placed at 16.3% with findings associating it to unknown levels from consumption of finger millet diets. In the light of this, levels of thiocyanate in the dry, sprouted and soaked grains, fresh, fermented and cooked flour from the red and brown varieties of finger millet are reported following measurements using UV-VIS spectrophotometer. Levels of thiocyanate content in the finger millet ranged from 4.28±0.5 to 53.30±0.78 mg/kg. Significant differences were observed between the levels of the analyte in the grain, flour as well as between the processing methods studied ($p<0.001$). Although levels were within recommended levels by the World Health Organization, we support the potential risk of exposure to goiter with consumption of finger millet that may result from thiocyanate bioaccumulation. Results point to a need to carefully consider processing methods prior to consumption of *Eleusine coracana*.

Keywords: Finger millet, thiocyanate, goiter

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Introduction

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). 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). 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). 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).

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).

Finger Millet (*Eleusine* coracana) 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).

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).

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. 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). 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.

Materials and methods

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. Random sampling was used to select the farmers in Mogotio. Sampling of the grains was done in 2012 and 2013 between the months of October and December during harvesting time. This was done two times every month during the three months. A bout 4.0 kg each of the red and brown finger millet grains was sampled then put in different plastic bags which were labeled well and taken to Kenyatta University, Chemistry laboratory. Analytical grade chemicals were used in the analysis. The chemicals included potassium thiocyanate (KSCN), de-ionized water, HNO_3 (65 % w/v), Trichloroacetic acid, saturated bromine water, Arsenous trioxide, pyridine, benzidine/phenyl diammine and hydrochloride. Thiocyanate content in the samples was determined using a UV−VIS spectrophotometer.

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.

Results

Thiocyanate levels in the grains

Table 1: Mean levels of thiocyanate (mg/kg) in the treated grains

Variety/Treatment	Concentration in (mg/kg)		P-value
	Red Mean±SE (Range)	Brown Mean±SE (Range)	
Fresh dried n=8	43.48 ± 1.56^b (39.11-47.85)	31.83 ± 1.88^b (26.57-37.09)	0.471
Sprouted n=8	39.93 ± 0.89^b (37.44-42.42)	53.30 ± 0.78^c (51.12-55.48)	<0.001

Soaked n=24	10.5±1.73[a] (2.02-18.98)	9.73±1.72[a] (1.31-18.15)	0.718
P-value	<0.001	0.015	

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 1, 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 (p<0.001). Sprouted treatment showed a significant difference between the brown variety and the red variety (P<0.001 at 95% confidence level). Sprouting significantly increased SCN in brown finger millet (P<0.001) while it did not significantly reduce SCN in Red finger millet (p>0.05).

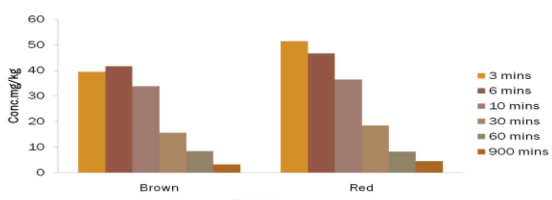

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Figure 1: variation of thiocyanate level with soaking time

Varying soaking time significantly reduced the levels of thiocyanate ($p<0.001$), with longer soaking time (900 minutes) reducing thiocyanate content to low levels.

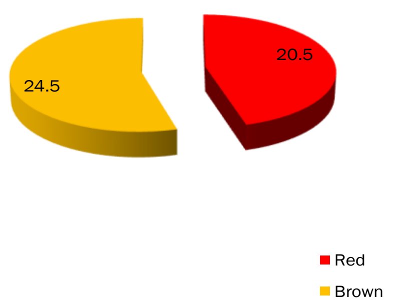
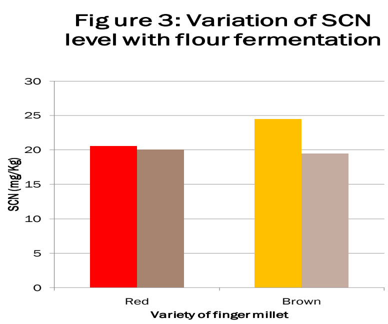


Figure 2: Thiocyanate level in fresh flour (mg/kg)

Levels of thiocyanate found in fresh flour were within safe levels, though there was no significant difference between the varieties.



Fermentation generally reduced levels of thiocyanate but there was no significant difference between the varieties.

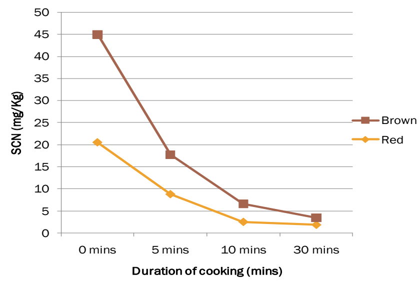


Figure 4: variation of thiocyanate with cooking of finger millet flour

Discussion

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 reduction due to soaking 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. . Cooked flour had the lowest thiocyanate level, followed by fermented then fresh flour in both the varieties. 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). 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^{0}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).

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