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Chemistry

Concentration of Thiocyanate in the Green and Dry Leaves of the Red and Brown Finger Millet from Baringo County of Kenya

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Original Research Article

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Abstract: Finger millet (*Eleusine coracana*) crop contains cyanogenic glycosides, which can be readily converted to thiocyanate by glycosidases, and sulfur transferase enzymes present in the plant and transferred in the animal tissues if they feed on leaves or grains of the plant. The leaves of the crop are eaten by animals, which provide meat and milk, introducing thiocyanate to the people. 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, which in the event of a nuclear accident and hypothyroidism during pregnancy 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%. It was therefore important to determine the levels of thiocyanate in the leaves. Drying is considered as one of the methods of processing millet that could reduce levels of thiocyanate in the leaves and the grains for animal consumption. 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. These levels of content found were within the recommended levels (100 mg/kg) but the frequency of ingestion may still result into health risks in the study area. It is therefore advocated that processing prior to cooking be encouraged as this reduces thiocyanate levels.
Keywords: thiocyanate, goiter, glycosidases, sulfur transferase enzymes, cyanogenic glycosides.

INTRODUCTION

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.

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 [1].

Sun drying facilitates the continuation of the fermentation process [2]. The residual level of thiocyanate in processed food would therefore depend on the processing method [3]. Cooking destroys active enzymes involved in thiocyanate formation at about 72°C leaving a considerable portion of glycoside intact [4]. Heat treatment negatively affects glucosinolates content, wet heating/pressure cooking is more effective over dry heating [5]. 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 [6].

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 [7]. 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 [8]. Thiocyanate in plants foods could be bound, free, or volatile. Each of these forms of thiocyanate responds 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 [9].

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 [10]. The experiment shows that pounding and allowing standing 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 [11].

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 [12]. The development of goiter is critically related to the balance between iodine and thiocyanate, a goitrogen found in some African diets [13]. 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 [14]. Consumption of naturally occurring goitrogens, certain environmental toxins, and cigarette smoking can significantly increase SCN^- concentrations to levels capabable 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^- [15].

Thiocyanate content in food plants

Studies by Chandra *et al*. [16] and Malik *et al.* [17] reported the levels of thiocyanate in cassava, cabbage, cauliflower, mustard, rape kale, beans, sweet potatoes, and carrots. Gaitan *et al.* [18] 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 [19].

Table-1: Thiocyanate content (mg/kg) in selected food plants

Plant	Levels (mg/kg)	Reference
Cauliflower	42.3±3.26	Malik *et al*., 2012
Cabbage	23±2.06	Chandra *et al*., 2004
Sweet potato	20.5±0.7	Chandra *et al*., 2004
Carrot	16.5±1.04	Malik *et al*., 2012
Kale	159	Chweya, 1990
Mustard	50±2.9	Chandra *et al*., 2004
Cassava	12.95±2	Chandra *et al*., 2004
Pearl millet	35	Gaitan *et al*., 1989

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 [20]. 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 [21]. A study on sorghum showed that level of thiocyanate precursors (HCN) are affected by age, genotype, temperature, phosphorus nutrition, and possibly light intensity [22]. 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 until maturity in order to feed their animals with sorghum [23].

METHODOLOGY

Sample preparation

Finger millet grains were cleaned by winnowing to remove dust and other extraneous materials. Unviable and broken grains were handpicked.

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 [24].

MATERIALS AND METHODS

The experimental design involved sampling of the red and brown finger millet, sample processing which were dryed and analysis of thiocyanate levels done. 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 drying. 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

Recovery test

The percentage recovery was calculated using equation 4.1 [25].

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

Where
SSR− Spiked sample result
USR− Unspiked sample result

The percentage recoveries from the spiked sample (Table 1) ranged between 90 – 99.80%, while RSD (3.65-5.01%) which was within the acceptable range for thiocyanate [26]. This confirms that the method is of good precision and fit for analysis of the above parameter.

Table-2: Percentage recoveries of thiocyanate

Test sample	% recovery	% RSD
Fresh dried grain	98.91	4.02
Sprouted grain	92.97	5.01
Fresh flour	99.80	3.65
Cooked flour	95.65	4.21
Green leaves	90.01	5.00

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 2 and table 3.

Table-3: 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

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 below.

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, 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).

Table-4: Mean levels of thiocyanate (mg/kg) in the green and dry leaves of the red and brown finger millet

Treatment	Red	Brown
	Mean±SE (Range) n=8	Mean±SE (Range) n=8
Green leaves	30.78±0.40[b] (28.88-32.56)	31.69±0.71[b] (28.80-34.05)
Dried leaves	9.00±0.13[a] (8.35-9.4)	8.80±0.14[a] (8.25-9.15)
p-value	<0.001	<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 [27]. 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 [28].

CONCLUSION

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

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