Journal of Food Science and Engineering 3 (2013) 648-656

Cyanide Contents of Leaves of Commonly Consumed Cassava Varieties from Three Geographical Regions of Ghana

William Opoku-Nkoom, Ebenezer Asibey-Berko and Anna Lartey

Department of Nutrition and Food Science, University of Ghana, Legon, Accra 233, Ghana

Received: April 3, 2013 / Published: December 20, 2013.

Abstract: Consuming a cyanogenic plant is an etiological factor to the persistence of iodine deficiency in the post salt iodization phase. Ghana, notably the Northern belt, still reports of iodine deficiency after 14 years of mandatory consumption of iodized salt by an Act of Parliament. The study aimed at determining the cyanide contents of leaves of commonly consumed cassava varieties in Ghana and investigating the effects of some environmental factors on cyanide content. Three communities each from Southern, Middle and Northern Ghana served as the study sites from where young, non-diseased and fully-spread cassava leaves were sampled from plants of commonly consumed cassava varieties. Cyanide was analyzed by the standard colorimetric method based on the chloramine-T/pyridine-barbituric acid protocol (4500-CN E). Cassava leaves from Northern Ghana had significantly higher mean cyanide content (177.22 ± 20.82 ppm) than those from Middle (130.83 ± 33.00 ppm) and Southern Ghana (127.24 ± 37.54 ppm) ($P < 0.001$). Two-factor ANOVA showed significantly higher adverse environmental effects on cyanide contents of leaves of unimproved cassava varieties than improved ones ($R^2 = 0.627$, $P = 0.023$). From multiple regression analysis, temperature was the most significant environmental factor explaining 33% of the variability in cyanide content ($R^2 = 0.331$, $P = 0.002$), followed by altitude ($R^2 = 0.106$, $P = 0.049$) and rainfall ($R^2 = 0.084$, $P = 0.062$). The high cyanide contents of cassava leaves from Northern Ghana, due principally to the high atmospheric temperature, may be a contributory factor to the high prevalence of goiter and the persistence of iodine deficiency in that geographic region.

Key words: Cassava leaves, cyanide content, iodine deficiency, Ghana.

1. Introduction

Cassava leaves are rich in protein (crude protein is 7.4 g/100 g), especially in the young apical leaves, which are the most appropriate for vegetable [1]. Even though cassava leaves are limiting in the sulphur-containing amino acids (methionine and cysteine), when consumed with cereals such as maize, rice and wheat staples (which are rich in the sulphur-containing amino acids but deficient in lysine), a good complementary effect is produced. Cassava leaves are significantly high in alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, lysine, proline, threonine and tyrosine [2]. Cassava leaves are also rich in micronutrients. For example, the β-carotene, iron and zinc contents of cassava leaves are 20,803.0 μg/100 g, 5.6 mg/100 g and 5.0 mg/100 g, respectively [1].

By virtue of their nutritional value, cassava leaves have the potential to address protein and micronutrient deficiencies in hungry populations, as noted specifically for vitamin A deficiency by some investigators [3, 4]. This potential is further enhanced by the fact that cassava cultivation is relatively cheaper, being drought-tolerant and its ability to do well in less fertile soils. Also, cassava leaves are readily affordable and supply may extend over longer periods in the course of the year. For these reasons, reliance on

Corresponding author: William Opoku-Nkoom, M.Phil., research field: nutritional toxicology. E-mail: william_nkoom@live.co.uk.

cassava (leaves) is expected to increase in the coming years, in the context of the global climate change [5]. Cassava leaves may therefore be considered a very important plant source of micronutrients and protein especially in populations which are hard-to-reach with fortified foods. Given these nutritional potentials, some authors have long advocated cassava leaves be made into acceptable edible forms through industrial means and backed up with educational programs to promote their consumption [6]. In Ghana, cassava leaves are being consumed by some segments of the population, most notably Northern Ghana.

One constraint with the nutritional potential or the consumption of cassava leaves is the presence of cyanogenic glycosides which release cyanide. One of the pathological disorders that have been associated with cassava cyanide consumption is iodine deficiency, especially in the form of goiter. This is because the major cyanide metabolite, thiocyanate is a known goitrogen. Goitrogens take their name from goiter and inhibit the uptake of iodine by the thyroid gland. As a result, the gland is not able to form thyroid hormones. Some goitrogens act non-competitively and cannot be neutralized by taking extra iodine. These are called goitrins and may be found in turnips and some cabbages [7]. The goitrogen thiocyanate, however, acts competitively. It competitively inhibit iodide uptake in the thyroid gland at 100 μmol/L of blood [8]. At this level, thiocyanate disrupts homeostatic feedback mechanisms that control biosynthesis, secretion and transport of thyroid hormones, leading to iodine deficiency, predominantly in the form of goiter.

In Ghana, iodine deficiency is a problem precisely in Northern Ghana and some mountainous areas of the country. In 1994, goiter was reported endemic in Northern Ghana [9]. Exactly, a decade of mandatory consumption of iodized salt by an Act of Parliament, iodine deficiency in Northern Ghana was reported to have improved but to the mild stage [10]. Most recent data on iodine nutrition in Ghana depict that iodine deficiency still persists in Northern Ghana despite the use of iodized salt in households [11].

From literature, consuming a cyanogenic plant is an etiological factor to the persistence of residual goiter in the post salt iodization phase [12]. The study therefore aimed at determining the cyanide contents of the leaves of most commonly consumed cassava varieties from Southern, Middle and Northern Ghana and investigating the effects of some environmental factors (altitude, rainfall, relative humidity and temperature) on cyanide concentration.

2. Materials and Methods

2.1 Selection and Description of Study Sites

Three Agricultural Research Stations (ARS), one each from the South, Middle and North of Ghana were selected. These were based on the fact that they were the most important ARS within the root and tuber improvement and marketing program (RTIMP) in Ghana. These were the Asuansi ARS, Wenchi ARS, and Damongo Agricultural Settlement (Fig. 1).

Asuansi ARS is located in the Abura-Asebu-Kwamankesi district in the Central Region (Southern Ghana) and is situated in the moist semi-deciduous forest agro-ecological zone (Fig. 1). Wenchi ARS is in the Wenchi district of the Brong-Ahafo Region (Middle belt) and is sited within the transition agro-ecological zone (Fig. 1). Damongo Agricultural Settlement is not an ARS but a place where there is commercial production of cassava, within the West Gonja district of the Northern Region (Northern Ghana). The West Gonja district is the leading producer of cassava in Northern Ghana [13]. This site is located in the guinea savanna agro-ecological zone. The South, Middle and the North belts were considered in order to obtain a wider scope as some varieties as *Filindiakong*, *Eskamaye* and *Nyerikobga* popularly known as the Northern improved varieties, are documented to thrive only in the guinea savanna zone [14].

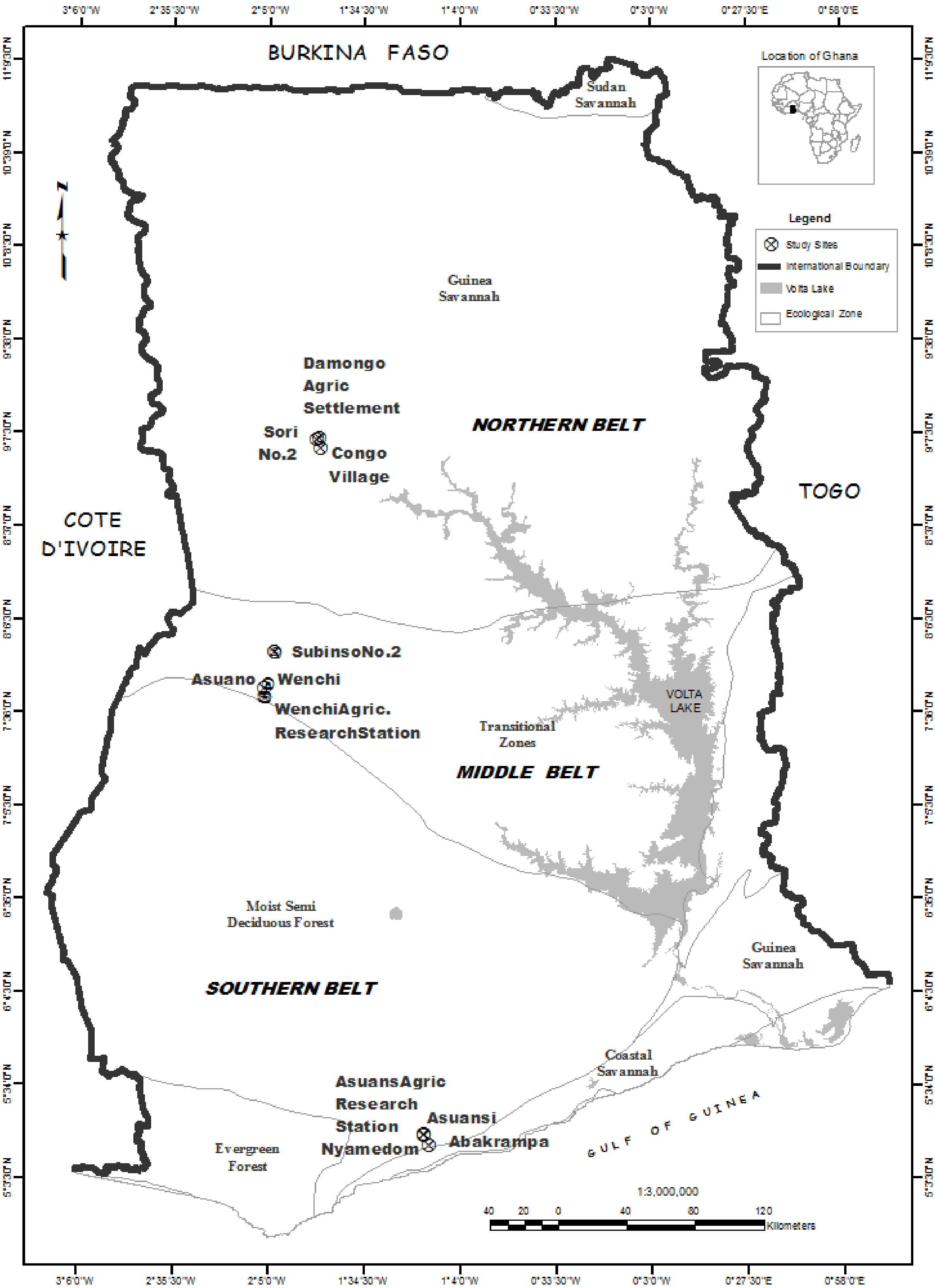

Fig. 1 Surveyed areas in the South, Middle and North of Ghana.

2.2 Sample Size and Selection of Respondents

The population under survey was cassava farmers. A total of 100 cassava farmers were interviewed on cassava varieties they were growing. This was weighted as follows: there were 12,818, 14,255 and 7,695 cassava farm holders in the Abura-Asebu-Kwamankesi district (Southern Ghana), Wenchi district (Middle Ghana) and West Gonja District (Northern Ghana), respectively [15]. Proportion-wise, 37, 41 and 22 cassava farmers were interviewed in each geographical region on most commonly consumed cassava varieties.

2.3 Sampling and Sample Collection

In each belt, farmers who satisfied inclusion/exclusion criteria (Section 2.4) were randomly selected to provide cassava leaves. For a single cassava variety (primary sample) in each belt, three sub-samples were obtained as shown in Fig. 2, using a semi-structured questionnaire. In the farm, leaves were obtained from randomly selected non-diseased plants. Leaves were collected from eight month-old (completed months) plants only. Young, tender and fully-spread cassava leaves, numbered 1-2 from the uppermost leaves of the randomly selected plants were harvested.

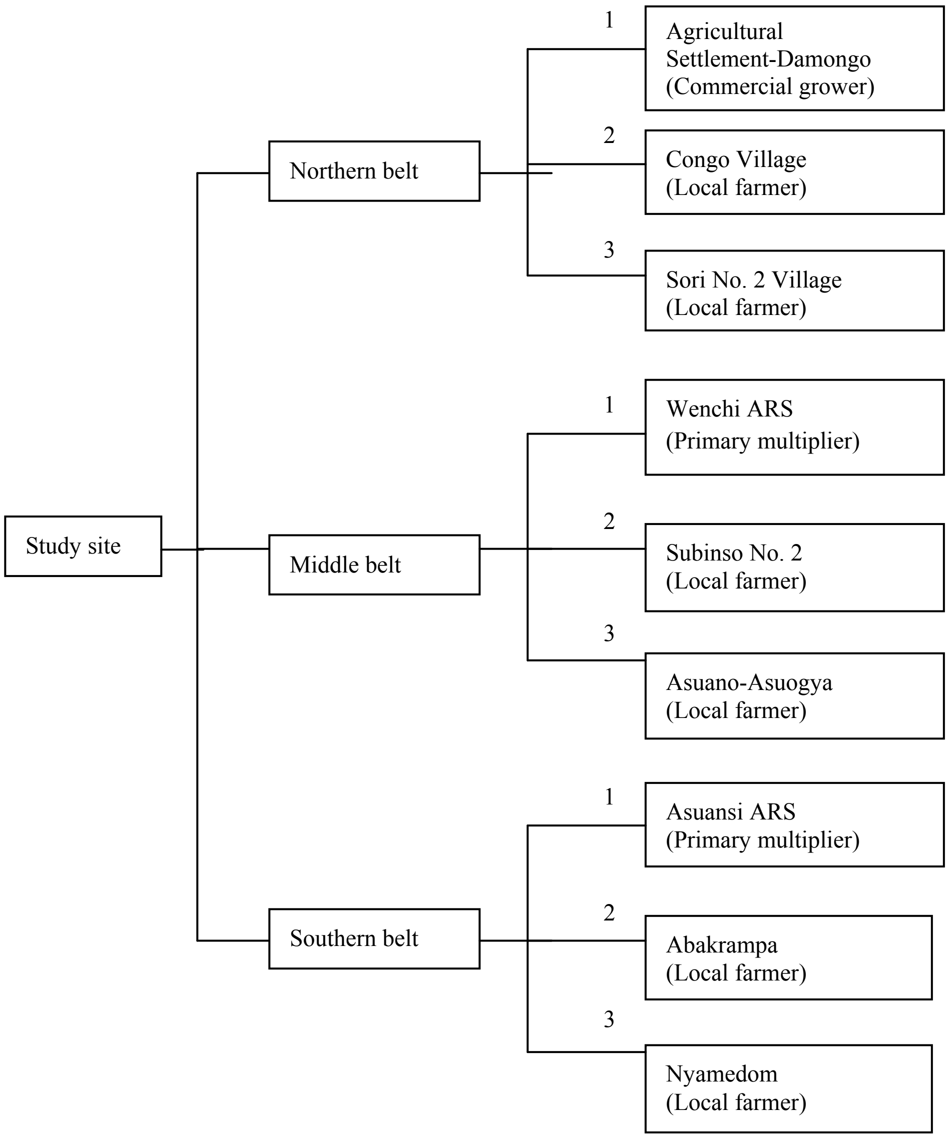

Fig. 2 Sampling procedure for collecting cassava leaves.

The leaves were immediately put in labeled plastic bags and transported on ice packs to the laboratory. Global positioning system (gecko 201, Garmin) was used to determine the altitudes and the exact locations of the farms where samples were taken.

2.4 Exclusion/Inclusion Criteria for Selecting Farmers

Without any external influences, the contents of cyanogenic glycosides in cassava increases to a peak level at three months, and then decreases to a constant level at six months and throughout the growth period [16]. Farmers who provided leaves were therefore those who could give exact information on plant age.

Selected farmers did not intercrop their cassavas with groundnuts, beans or any other leguminous crops, in order to rule out any interferences of nitrogen fixation since this is known to be one of the most important exogenous factors and the number one soil nutritional factor that influences the actual level of cyanogenic glycoside or cyanide level of plants [17].

Selected farmers did not apply nitrogen, phosphorus, potassium or any other inorganic fertilizers in the farm since this is known to influence cassava cyanogenesis [18, 19].

2.5 Preparation of Analytical Samples

In obtaining food samples in the field for chemical analysis, it is suggested that, besides analysis on each primary sample obtained for the same type of sample in the same geographical location, a composite sample could also be prepared from primary samples of the same sample type [20]. In accordance with this, composite samples were prepared from the three primary samples obtained for each sample type in each belt. For each leaf, the petiole was removed. Cyanide content of cassava leaves is found to increase from the tip to the stalk end [21]. Therefore, in order to obtain representative samples for analysis, only the central portions of the leaves were used. These central portions were cut into smaller pieces and quickly ground on ice packs using pre-chilled glass mortar and pestle until a homogenous marsh was obtained. Each composite sample (homogenous mash) was quickly transferred into a labeled plastic bag and kept at -4 °C until analysis.

2.6 Determination of Cyanide Concentration

Cyanide analysis was done using the standard colorimetric method based on the chloramine-T/pyridine-barbituric acid protocol (4500-CN E) at the Société Générale de Surveillance (SGS) laboratory in Tema-Ghana [22]. To avoid cyanide loss from pounded cassava leaves, due to the high enzymatic catalytic activity at room temperature [21, 23], weighing the analytical sample prior to analysis was done as quickly as possible. Analyses were done in duplicates.

2.7 Data Analysis

Climatic data (temperature, rainfall and relative humidity) for the year 2010 (the year when the cassavas were cultivated) for each belt, were obtain from the Ghana meteorological agency.

One-way ANOVA was used to determine whether any significant differences in cyanide concentration existed in the three geographical belts. Multiple regression analysis was used to determine the potential environmental factors that predict cyanide concentration. Two-way ANOVA was used to study the interactive effects of environmental factors (geographical location) and type of variety (improved/unimproved).

3. Results and Discussion

3.1 Cyanide Contents of Leaves of Most Commonly Consumed Cassavas

Nine cassava varieties were found to be most commonly consumed (i.e., $\geq$ 5% of the respondents were growing the variety, and therefore important). Five were improved, four were unimproved (Table 1).

Table 1 Moisture and cyanide contents of the leaves of most commonly consumed Ghanaian cassava varieties (duplicate analyses).

Name of variety	Mean moisture content (Mean ± SD) (%)	Dry matter mean cyanide (Mean ± SD) (ppm)
[a]*Afisiafi* (IV-SB)	87.20 ± 0.28	107.06 ± 2.36
Bankyehemaa (IV-SB)	70.36 ± 0.43	175.82 ± 2.52
[a]*Bosomensia* (UV-SB)	70.50 ± 0.71	99.97 ± 2.40
Afisiafi (IV-TB)	75.90 ± 0.42	72.79 ± 1.11
[b]*Afosa* (UV-TB)	72.90 ± 0.42	140.85 ± 2.21
[b]*Bankyehemaa* (IV-TB)	71.30 ± 0.71	144.53 ± 3.56
Bensere (UV-TB)	72.85 ± 0.28	165.81 ± 1.73
Capevas Bankye (IV-TB)	76.36 ± 0.42	131.64 ± 2.33
Afisiafi (IV-NB)	73.10 ± 0.71	144.42 ± 2.69
[c]*Bosomensia* (UV-NB)	73.70 ± 0.14	187.64 ± 1.01
[c]*Eskamaye* (IV-NB)	71.90 ± 0.71	181.01 ± 4.55
Gbanfuful (UV-NB)	74.90 ± 0.42	203.50 ± 3.44
Nyerikogba (IV-NB)	71.20 ± 0.57	171.20 ± 3.37

IV = Improved variety; UV = Unimproved variety; SB = Southern belt; TB = Transition belt; NB = Northern belt. Same superscripts do not show significant difference in mean cyanide concentration.

As depicted in Fig. 3, one-way ANOVA showed the mean cyanide content of varieties in the Southern belt (127.24 ± 37.54 ppm), Middle belt (130.83 ± 33.00 ppm) and the Northern belt (177.22 ± 20.82 ppm) to be significantly different (P = 0.003). Post-hoc analysis, however, revealed no difference between the Southern and the Middle belts in mean cyanide concentration.

3.2 Impacts of Environmental Factors on Cyanide Contents

Investigation into finding what factors may plausibly explain the results revealed temperature as the most significant environmental factor predicting cyanide concentration from multiple regression analysis (R^2 = 0.331, P = 0.002), followed by altitude (R^2 = 0.106, P = 0.049), and then rainfall (R^2 = 0.084, P = 0.062). Overall, about 52% (R^2 = 0.521, P < 0.001) of the variability in cyanide concentration was explained by these environmental factors.

Of note, is the high temperature in the Northern belt of Ghana. The average temperature recorded in that geographical region was 29.28 °C (range: 21.4-38.1 °C). This significantly differed (P < 0.001) from that of the transition belt (mean: 26.97 °C, range: 20.3-35.4 °C), and the Southern belt (mean: 27.97 °C, range: 23.1-33.8 °C). One degree rise in temperature significantly (P = 0.002) increased cyanide level by 20.71 ppm. The high atmospheric temperature in the Northern belt of Ghana may therefore explain the high cyanide contents of the cassava varieties found there. This is consistent with literature as some investigators have reported greater frequency of *Trifolium repens* (another cyanogenic plant) at high temperatures in the United States [24]. Our findings justify the question posed by some researchers, as to whether cassava (leaves) would continue to be safe under the influence of the global climate change, which is characterized by high atmospheric temperatures, as reliance on this crop increases in tandem [25].

High incidence of konzo (one of the pathological effects of cassava cyanide consumption) has been reported during periods of drought (lack of rainfall events) in Democratic Republic of Congo and Mozambique [26, 27]. According to the authors, this was due to increased cyanogenic potential in cassava products during periods of drought. From our data, rainfall correlates negatively with cyanide (r = -0.192), and 1 mm increase in rainfall reduced cyanide concentration by 0.49 ppm even though this was not found statistically significant. Rainfall patterns in Ghana show minimal rainfall in the Northern belt (1,159.1 mm annual rainfall) compared to 1,459.0 mm

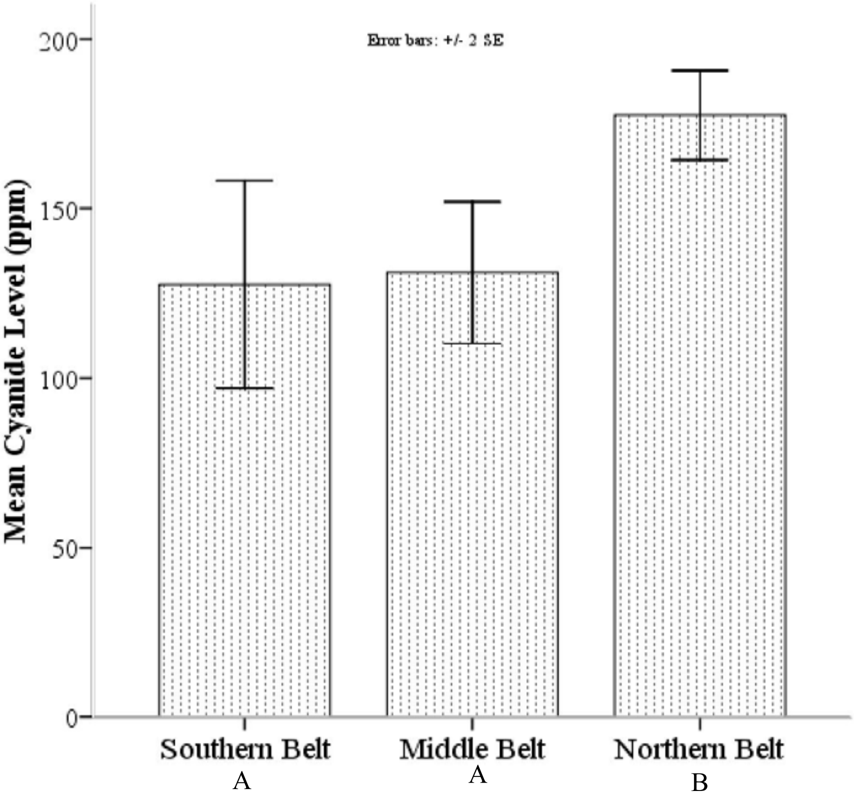

Fig. 3 Mean cyanide concentration of cassava leaves by geographic region.

in the transition belt and 1,218.20 mm in the Southern belt. These may further help explain the higher cyanide concentration in the varieties of the Northern belt, and are supportive of the high cyanogenetic frequency of *Trifolium repens* reported by the US investigators at low summer precipitation [24].

High cyanogenic cassavas have been reported at low altitudes in Nigeria in places of endemic tropical ataxic neuropathy, a neurological disease attributable to monotonous cassava (cyanide) consumption; whereas low cyanogenic cassavas have been reported in Tanzania at locations of high altitudes [28]. Consistent with our results, 1 m increase in altitude above sea level was associated with 0.52 ppm decrease in cyanide concentration, though not statistically significant. Moreover there was a strong correlation between altitude and rainfall ($r = 0.99$, $P < 0.001$). High rainfall is therefore expected in places of high altitudes. This may explain why the transition belt, the location with the highest average altitude (287.95 ± 27.04 m above sea level, compared to 168.27 m in the North and 100.22 m in the South) had the highest rainfall pattern (annual rainfall = 1,459.0 mm compared to 1,218.2 mm in the South and 1,159.1 mm in the North). High altitude and high rainfall in the transition belt could therefore be the reason for the 32% and 18% respective decreases in cyanide levels of *Afisiafi* and *Bankyehemaa* in the Southern belt when the same varieties were located in the transition belt (Table 1). The transition belt could thus be thought as the safest zone with respect to cassava cyanogenesis.

3.3 Gene-Environment Interaction

Two-way ANOVA showed significant interaction between geographical location and type of variety (unimproved/improved) ($P = 0.023$, $R^2 = 0.627$). What this suggests is that the impact of the environment on cyanide level is dependent on the type of variety, whether improved or unimproved. In other words, depending on the environmental exposure, improved or unimproved variety would accumulate more cyanogenic glycosides than the other. This is clearly depicted in the data (Table 1). It was found that the unimproved varieties accumulated more cyanogenic glycosides, as depicted in their mean cyanide contents (improved: 141.06 ± 35.93 ppm; unimproved: 159.55 ± 38.51 ppm). Of note is the fact that this difference was not significant statistically but when stratified by geographical location, it was found significant in the Northern belt (P = 0.014).

To shed more light on this, *Afisiafi* (improved variety) in the Southern belt recorded cyanide concentration of 107.06 ± 2.36 ppm; the same *Afisiafi* in the Northern belt increased cyanide concentration by 35% (144.42 ± 2.69 ppm). Under the same prevailing environmental conditions, *Bosomensia* (unimproved variety) increased cyanide concentration by 88% (99.72 ± 2.39 ppm, in the Southern belt and 187.36 ± 0.98 ppm, in the Northern belt). It is documented that genotype-environment interaction could be very strong to the extent that if a particular variety finds itself under different environmental conditions; cyanogenic potential could increase up to five folds [29]. This extent of increase was not recorded in this study, but close to 90% increase in cyanogenic potential is very significant. This further suggests that the genotype-environment interaction in Ghanaian cassavas may have more adverse effects on the unimproved varieties compared to the improved ones.

4. Conclusions

The mean cyanide content of cassava leaves from the Northern belt of Ghana was significantly higher than that from the Middle and the Southern belts. Temperature was found to be the most significant environmental factor responsible for high cyanogenesis in cassava leaves. The impact of environmental factors on cassava cyanogenesis was found to be stronger (pointing to higher accumulation of cyanogenic glycosides) in the unimproved varieties compared to the improved ones. The high cyanide content of cassava leaves from Northern Ghana due principally to the high atmospheric temperature may be a contributory factor to the high prevalence of goiter and the persistence of iodine deficiency in that geographic region of Ghana.

Acknowledgments

The abstract of this paper was presented in the African Nutrition Congress 2012 at the University of Free State, Bloemfontein, South Africa. The authors are grateful for funding provided by the International Development Research Center (IDRC, Canada) and the Double Fortified Salt Project.

References

[1] B. Stadlmayr, U.R. Charrondiere, P. Addy, B. Samb, V.N. Enujiugha, R.G. Bayyli, et al., Composition of selected foods from West Africa, Food and Agriculture Organization, Rome, 2010, pp. 13-14.

[2] D.D. Ngudi, Y.H. Kuo, F. Lambien, Amino acid profiles and protein quality of cooked cassava leaves or “saka-saka”, J. Sci. Food Agric. 83 (2003) 529-534.

[3] E.M.A. Siqueira, S.F. Arruda, R.M. Vargas, E.M.T. Souza, β-carotene from cassava (*Manihot esculenta* Crantz) leaves improves vitamin A status in rats, Comparative Biochemistry and Physiology Part C 146 (2007) 235-240.

[4] E.E.K. Takyi, Children’s consumption of dark green, leafy vegetables with added fat enhances serum retinol, J. Nutr. 129 (1999) 1549-1554.

[5] A. Burns, R. Gleadow, J. Cliff, A. Zacarias, T. Cavagnaro, Cassava: The drought, war and famine crop in a changing world, Sustainability 2 (2010) 3572-3607.

[6] P.A. Lancaster, J.E. Brooks, Cassava leaves as human food, Economic Botany 37 (1983) 331-348.

[7] F.M. Strong, Toxicants occurring naturally in foods, in: Present Knowledge in Nutrition, 4th ed., Nutrition Foundation Inc., NY, 1976, p. 516.

[8] V.R. Preedy, G.N. Burrow, R. Watson, Comprehensive Handbook of Iodine: Nutritional, Biochemical, Pathological and Therapeutic Aspects, Elsevier Inc., Oxford, 2009, p. 275.

[9] E. Asibey-Berko, R. Orraca-Tetteh, Proceedings for the National Workshop on Iodine Deficiency Disorders in Ghana, 1994.

[10] E. Asibey-Berko, J.G.A. Armah, Biological impact assessment study on salt iodization program in Ghana (Jirapa and Bongo sentinel districts), UNICEF, 2007, pp. 1-71.

[11] S. Newton, E.F. Amoaful, E. Quaye, F. Rohner, C. Clewes,

J. Knowles, Iodine nutrition in Ghana, regional differences persists 14 years after legislation for universal salt iodisation, in: Proceedings of the Nutrition Congress Africa, Bloemfontein, South Africa, 2012.

[12] R.K. Marwaha, N. Tandon, N. Gupta, A.K. Karak, K. Verma, N. Kochupillai, Residual goitre in the post-iodisation phase: Iodine status, thiocyanate exposure and autoimmunity, Clinical Endocrinol 59 (2003) 672-681.

[13] SRID-MoFA, Statistical research and information directorate, Ministry of Food and Agriculture, Ghana, 2010.

[14] RTIMP, Cassava production guide: A resource and reference manual, root and tuber improvement and marketing programme, MoFA, 2004, pp. 3-5.

[15] SRID-MoFA Statistical Research and Information Directorate, Ministry of Food and Agriculture, Ghana, 2008.

[16] B. Nambisan, S. Sundaresan, Distribution pattern of cyanoglucosides in cassava tubers and leaves at different growth stages, J. Root Crops 17 (1991) 261-263.

[17] J. Vetter, Plant cyanogenic glycosides, Toxicon 38 (2000) 11-36.

[18] B. Nambisan, Strategies for elimination of cyanogens from cassava for reducing toxicity and improving food safety, Food and Chemical Toxicology 49 (2011) 690-693.

[19] S. Endris, Cyanogenic potential of cassava cultivars grown under varying levels of potassium nutrition in South-Western Ethiopia, Gene Conserve 7 (2008) 438-445.

[20] R.S. Gibson, Ferguson, An interactive 24-hour recall for assessing the adequacy of iron and zinc intakes in developing countries, Harvest Plus Technical Monograph 8 (2008) 86.

[21] J.H. Bradbury, I.C. Denton, Mild methods of processing cassava leaves to remove cyanogens and conserve key nutrients, Food Chemistry 127 (2011) 1755-1759.

[22] APHA Standard Colorimetric Method for Cyanide (4500-CN E), 21st ed., American Public Health Association, 2005.

[23] A.M.B. Priyadarshani, E.R. Jansz, H. Peiris, S. Jayasinghe, Detoxification of cassava leaves, J. Natn. Sci. Foundation Sri Lanka 32 (2004) 61-68.

[24] G.A. Pederson, T.E. Faitbrother, S.L. Greene, Cyanogenesis and climatic relationships in US white clover germplasm collection and core subset, Crop Science 36 (1996) 427-433.

[25] A. Burns, R. Gleadow, J. Cliff, T. Cavagnaro, Effects of increasing carbon dioxide emissions and climate change on nutritional quality of food crops: A case study on cassava, SCN News 38 (2010) 49-53.

[26] J.P. Banea-Mayambu, T. Tylleskär, N. Gitebo, N. Matadi, H. Rosling, Geographical and seasonal association between linamarin and cyanide exposure from cassava and the upper motor neuron disease konzo in former Zaire, Tropical Medicine and International Health 2 (1997) 1143-1151.

[27] A.P. Cardoso, M. Ernst, J. Cliff, H. Bradbury, High levels of total cyanogens in cassava flour related to drought in Mozambique, Roots 6 (1999) 4-6.

[28] O.S. Oluwole, A.O. Onabolu, K. Mtunda, N. Mlinga, Characterisation of cassava (*Manihot esculenta* Crantz) varieties in Nigeria and Tanzania, and farmers' perception of toxicity of cassava, Journal of Food Composition and Analysis 20 (2007) 559-567.

[29] M. Bokanga, I.J. Ekanayake, A.G.O. Dixon, M.C.M. Porto, Genotype-environment interactions for cyanogenic potential in cassava, Acta Hort. 375 (1994) 131-139.