Bioscience Research Communications
Vol. 15, No. 3, June 30, 2003
Printed in Nigeria

0795-8072/2003 $12.00 + 0.00

http://www.klobexpublishers.com

BRC 2002068/15308

An evaluation of cyanoglucose, total phenol, protease inhibitors, phytic acid and zinc contents in some Caribbean food crops

F. O. Omoruyi[1]; L. Dilworth[1]; H. N. Asemota*[1,2]; H. Jacobs[3] and E. Y. Morrison[1]

[1]Dept. of basic Medical Sciences (Biochemistry Section); [2]Biotechnology Centre and [3]Dept. of Chemistry, University of the West Indies, Mona, Kignston 7, Jamaica.

(Received September 17, 2002)

ABSTRACT: Six staple foods grown in the Caribbean (gungo peas, red peas, lettuce, callaloo, pumpkin and green banana) were screened in uncooked form for their contents of cyanoglucosides, phenolics, phytic acid, protease inhibitors and zinc.

Cyanoglucoside content was lowest for red peas and highest in callaloo (2.43 ± 0.22 and 4.26 ± 0.36 mg/100g dry weight respectively). Total phenol level was lowest in green banana (6.3 ± 1.40 µg/g dry weight) and highest in lettuce (122.20 ± 2.95 µg/g dry weight). Trypsin inhibitor activity was lowest for pumpkin (32.55 ± 2.45 TIU/g dry weight) and highest in gungo peas (185.50 ± 2.80 TIU/g dry weight). Chymotrypsin inhibitor activity was lowest in callaloo (9.10 ± 1.40 CIU/g dry weight) and highest for gungo peas (137.90 ± 2.19 CIU/g dry weight). Phytate content of the food crops was lowest for callaloo (8.27 ± 1.36mg/g dry weight) and highest for gungo peas (25.82 ± 3.00 mg/dry weight). The phytate: zinc ratio was higher than 10 for all the food samples analysed except for lettuce and callaloo that recorded Phytate: Zn of 8 and 4 respectively. The consumption of some of these food crops in the raw or improperly processed forms may elicit some adverse effects in humans and animals.

Key words: Cyanoglucoside, minerals, phytic acid, protease inhibitors, staple food, total phenol.

Introduction

The bulk of the food that we consume provides nutrients such as carbohydrates, lipids and proteins and a variety of vitamins and minerals to maintain proper function. In addition, there are compounds known as anti-nutritional factors, which act to reduce utilization of these valuable butrients. The World Health organization (WHO) in 1985 (1) reported that the West Indian countries have the highest rate of diabetes mortality among all ages. The prevalence of this disease in the Caribbean islands is high and is in the range of 15-20% of total population. Several studies have also shown that anaemia is a major health problem in Jamacia. Even in the midst of flour fortification with iron since 1984 in Jamaica, clinic data indicate that anaemia is still a major problem among antenatals.

*To whom correspondence should be addressed.

These health problems may be linked to the dietary pattern of the people. A few of the commonly eaten foods that have been analysed have been found to contain anti-nutritional components, which have been implicated in the aggravation of diabetes (2). Ragoobirsingh et al (3) have also reported a link between under-nutrition and the consumption of staples containing cyanide-yielding substances and diabetes.

Anti-nutritional factors are believed to be natural constituents of commonly eaten foods. Food crops regularly eaten contain traces of anti-nutritional factors such as cyanoglucosides, phytic acid, phenolics, trypsin and chymotrypsin inhibitors, heavy metals etc. that may have adverse effects on health through inhibition of protein digestion, growth, Fe and Zn absorption (4,5). A survey of the literature reveals that most food crops commonly eaten in the Caribbean are yet to be screened for anti-nutritional factors. These food crops may contain chemicals known to have toxic properties. This study was designed to determine the levels of some anti-nutritional factors in selected commonly eaten Caribbean food crops.

Materials and Methods

Fresh matured food crops, namely gungo peas (*Cajanus cajan*); red peas (*Phaseolus sp.*); lettuce (*Lactuca sativa*), callaloo (*Amaranthus sp.*); pumpkin (*Cucurbita moschata*), and green banana (*Musa sp.*) about 2-7 days after harvest were purchased from a local farmer in the Parish of St. Andrew, Jamaica. Each batch of samples was washed with distilled water, oven dried at 65°C to constant weight and ground into fine powder.

Cyanoglucoside assay: The level of endogenous cyanoglucoside was determined by the method of Ikediobi et al (6) in which the cyanide liberated by enzyme hydrolysis was quantified using alkaline picrate.

Total phenol: The phenolic compounds were extracted from 10g portions of dried powder sample with acetone (100%). The extract was dried and spectrophotometrically analysed at 725nm for total phenols as described by Swain and Hillis (7) using phenol standard.

Phytic acid determination: 0.5g of dried sample was extracted with 20ml 0.5M-HNO_3 for 3-4h with continuous shaking. After filtering, phytate analysis was performed on the filtrate by modification of Holt's (8) method as described by Davies and Redi (9). Sodium phytate (Sigma Chemical Co., St. Louis, Mo, USA) was used for the preparation of standard phytate solution.

Protease inhibitors: The method of Veerabhadrappa et al. (10) was used for the extraction of food samples. Sample flour (1.0g) was defatted by stirring with ten volumes of water-saturated n-butanol for 24h and filtered. The defatted flour was dried at room temperature and ground to a fine powder. The powder samples were stirred with 0.05M sodium phosphate buffer (pH 7.6) for 4h at 4°C using flour to solvent ratio 1:10 (w/v). The extracts were then centrifuged 4000rev min^{-1} for 15 min at 0°C. The clear supernatant was dialyzed against 0.05M phosphate buffer (pH 7.6) in the cold and used for the estimation of trypsin and chymotrypsin inhibitory activities by Casein digestion method (11). Enzyme solution (40µg of trypsin or 24µg of chymotrypsin) in 0.1M phosphate buffer (pH 7.6) was incubated at 37°C with casein. Incubation was for 20 min in the case of trypsin and for 10min in the case of chymotrypsin. The reaction was stopped by addition of 6.0ml of 5% trichloroacetic acid. The suspension was filtered, and the absorbance of the filtrate was read at 280nm (trypsin assay) or at 275nm (chymotrypsin assay).

For the assay of inhibitory activity, the enzyme (40µg of trypsin or 24µg of chymotrypsin) was pre-incubated at 37°C for 10 minutes with aliquots of the inhibitor extract and the residual enzyme activity was determined by the casein digestion method. One trypsin unit (TU) or chymotrypsin unit (Cu) is arbitrarily defined as an increase of 0.01 absorbance unit at 280nm or 275nm respectively, per 10ml reaction mixture. Trypsin inhibitory units (TIU) or chymotrypsin inhibitory units (CIU) are defined as the numbers of TU or CU inhibited under the same assay conditions.

Determination of minerals: Zinc was estimated by atomic absorption spectrophotometer (AAS) (12). Samples were dried in the oven at 150°C for 4hrs, milled and ground to a fine powder. A known weight of ground sample was weighed into a 100ml porcelain dish, placed in a cold furnace and the temperature

raised gradually to 500°C. Samples were ashed overnight, treated with 1.0ml of concentrated nitric acid and evaporated to dryness over hot plate. The residue was returned to the furnace and ashing continued for 1hr. 5ml of 20% HCl cover crucible and digested at low hear for about 15 minutes, cooled and made up 25ml with de-ionized water. Samples were read using AAS at 213.9mm with slit width of 0.7.

Results and Discussion

Table 1 shows cyanoglucoside and total phenol levels in selected Caribbean food crops. Cyanoglucoside content was highest for callaloo and the lowest was recorded for gungo peas. Cyanoglucoside level was least in red peas. Total phenol level was generally low in the selected Caribbean food crops. The level of total phenol was highest in lettuce and lowest in green banana.

Table 1: Cyanoglucoside and total phenol levels in selected Caribbean food crops.

	Cyanoglucoside (mg/100g dry weight)	**Total phenol (µg dry weight)**
Gungo peas	3.41 ± 1.10	26.00 ± 2.80
Red peas	2.43 ± 0.22	32.50 ± 8.50
Lettuce	2.61 ± 0.64	122.20 ± 2.95
Callaloo	4.26 ± 0.36	42.70 ± 1.60
Pumpkin	2.60 ± 0.23	49.00 ± 1.90
Green banana	2.83 ± 0.90	6.30 ± 1.40

Means ± SEM, n = 3.

Table 2 shows trypsin and chymotrypsin inhibitory units in selected Caribbean food crops. The food crops analysed also contain trypsin and chymotrypsin inhibitors. The highest trypsin inhibitory units were found in gungo peas followed by red peas.

Table 2: Trypsin and chymotrypsin activities and the respective percentage inhibition in selected Caribbean food crops.

	Trypsin (TIU[a]/g dry weight)	**Chymotrypsin (CIU[b]/g dry weight)**
Gungo peas	185.50 ± 2.80	137.90 ± 2.10
Red peas	152.60 ± 9.10	18.20 ± 3.50
Lettuce	52.50 ± 14.00	12.60 ± 3.50
Callaloo	40.25 ± 4.55	9.10 ± 1.40
Pumpkin	32.55 ± 2.45	22.75 ± 3.85
Green banana	40.25 ± 7.35	11.90 ± 2.80

[a]TIU = Trypsin inhibitory unit
[b]CIU = Chymotrypsin inhibitory unit
Means ± SEM, n = 3.

The trypsin inhibitory unit level was relatively low in the other food crops withy the lowest level recorded in pumpkin. Trypsin inhibitory unit level was generally highest in legume crops. Chymotrypsin inhibitory unit activity was also highest for gungo peas and lowest in callaloo. The presence of these anti-nutritional factors in most of the dietary food crops could affect the availability of nutrients from these foods. Protease inhibitors have been reported (13) to reduce the ability to effectively utilize dietary protein and are also known to cause pancreatic hypertrophy. The legumes assayed contain high activity of protease inhibitors, which may reduce the digestion of proteins from these foods through the inhibition of trypsin and chymotrypsin enzymes in the stomach. Veerabhadrappa et al., (10) reported that protease inhibitors are labile under appropriate heat treatment in finger millet and if this is the case with the legumes used in this study, proper processing of these legumes may not demonstrate adverse effects in humans but may be of concern if these legumes are used raw as source of protein in the formulation of animals' feeds. The isolation and characterization of these protease inhibitors need further studies. The effect of cooking on protease inhibitor activities in these Caribbean food crops is in progress.

Phytic acid content was highest in gungo peas, followed by green banana and pumpkin (Table 3). Phytic acid content was lowest in callaloo. The highest level of zinc was seen in callaloo, followed by lettuce and lowest in green banana. The ratios of phytate to zinc in all the food crops except callaloo and lettuce were above 10. Dietary phytate has been shown to reduce Zn availability in humans (14). Davies and Olpin (15) had earlier reported that phytate: Zn ratio of 10:1 can induce Zn deficiency in rats. This has been shown to significantly reduce plasma and hair Zn concentration. They also reported that values of 15:1 and greater demonstrated reduction in growth rates. This study showed much higher phtic acid: zinc ratio in all the food crops analysed except for lettuce and callaloo. Inccreased consumption of most of these food crops without adequate supplementation with dietary Zn supply may result in the manifestation of the above deleterious effects in humans/animals. Osagie et al., (16) had earlier reported that cooking or dehulling does not so easily reduce phytic acid level in foods. Simmons (17) study in Jamaica in 1990 reported poor absorption of iron supplements as inhibiting factor militating against iron utilization. Phytic acid has been reported (18, 19) to inhibit iron absorption. The phytic acid content in the Caribbean staple foods assayed is generally higher than those of Nigerian-grown staple foods (20), which could partly explain the prevalence of iron deficiency in Jamaica even in the midst of flour fortification with iron. This however needs further investigation.

In conclusion, the consumption of these food crops in the raw or improper processed forms may elicit some adverse effects in humans and animals. Isolation and characterization of the protease inhibitors in the legumes analysed may enhance their utilization in nutraceutics.

Table 3: Phytic acid and Zinc levels and the ratio of phytic acid to zinc in selected Caribbean food crops.

	Phytic acid (mg/g dry weight)	**Zinc (µg/g dry weight)**	**Phytic acid:Zinc (Molar ratio)**
Gungo peas	25.82 ± 3.00	65.34 ± 2.012	27
Red peas	14.64 ± 1.38	64.95 ± 1.3	16
Lettuce	12.70 ± 1.0	116.06 ± 1.2	8
Callaloo	8.27 ± 1.3	126.6 ± 8.09	4
Pumpkin	20.57 ± 1.9	36.65 ± 3.87	38
Green banana	21.34 ± 1.0	16.52 ± 0.34	88

Means ± SEM, n = 3.

References

1. World health Organisation (1985). World Health Organisation Group, Technical Report Series No. 727, Geneva.
2. Grindley, P.B., Omoruyi, F.O.; ASEMOTA, h.n.; Morrison, E.Y. (2000). Hyperglycaemia, hyperlipidaemia and liver lipid metabolism in streptozotocin-induced diabetic rats fed extracts of yam (*Dioscorea cayenensis*) and dasheen (*Colocassia esculenta*). Med. Sci. Res. 28(2): 127 – 130.
3. Ragoobirsingh, D.; Robinson, H.M.; Morrison, E.M. (1993). Effects of cassava cyanoglucoside, linamarin on blood sugar levels in the dog. J. Nutr. Biochem. 4: 625 – 629.
4. Liener, I.E.; Kakade, M.L. (1980). Protease inhibitors In: Toxic Constituebnts of Plants Foodstiffs, Liener, I.E. (ed.), New York, Academic Press.
5. Larsson, M.; Rossander-Hulthen, L.; Sandstrom, B.; Sandberg, A. (1996). Improved zinc and iron absorption from breakfast meals containing malted oats with reduced phytate content. Brit. J. Nutr. 76: 677 – 688.
6. Ikediobi, C.O.; Onyia, G.O.C.; Eluwah, C.E. (1980). A rapid and inexpensive enzymatic assay for total cyanide in cassava and cassava product. Agric. Biol. Chem. 44: 2803 – 2809.
7. Swain, T.; Hillis, W.E. (1959). The phenolic constituents of *Prunus domestica*. 1. The quantitative analysis of phenolic constituents. J. Sci. Food Agric. 10: 63 – 69.
8. Holt, R. (1955). determination of phytate phosphorus. J. Sci. Fd. Agric. 6: 136 – 142.
9. Davies, N.T.; reid, H. (1979). An evaluation of phytate, zinc, copper, iron and manganese contents of, and Zn availability from soya-based textured-vegetable-proptein meat-sunstitutes or meat-extenders. The Nutrition Society, Great Britain, pp. 579 – 588.
10. Veerabhadrappa, P.S.; Manjunath, N.H.; Virupaksha, T.K. (1978). Proteinase Inhibitors of Finger Millet (*Eleusine coracana Gaertn.*). J. Sci. Fd. Agric., 29: 353 – 358.
11. Kakade, M.L.; Simons, N.; Liener, I.E. (1970). Note on the determination of chymotrypsin and chymotrypsin inhibitor activity using casein. Analyt. Biochem. 33: 225 – 258.
12. AOAC (1980). Official Methods of Analysis, 13th and 15th edns. Washington, DC; Association of Official Analytical Chemists.
13. Gertler, A.; Birk, Y.; Bondi, A. (1967). A comparative study of the nutritional and physiological significance of pure soybean trypsin inhibitors and of ethanol-extracted soybean meals in chicks and rats. J. Nutr. 91: 358 – 370.
14. Reinhold, J.G.; Nasr, K.; Lahimgarzadeh, A.; Hedayati, H. (1973). Effect of purified phytate and phytate-rich bread upon metabolism of zinc, calcium, phosphorus and nitrogen in man. Lancet, 1: 283 – 288.
15. Davies, N.T.; Olphin, S.E. (1979). Studies on the phytate:zinc molar contents in diets as a determinant of Zn availability to young rats. Brit. J. Nutr. 41(3): 591 – 604.
16. Osagie, A.U.; Muzquiz, M.; Burbano, C.; Cuadrado, C.; Ayet, G.; Castano, A. (1996). Anti-nutritional constituents of ten staple foods grown in Nigeria. Trop. Sci. 36: 109 – 115.
17. Simmons, W.K. (1990). Evaluation of side effects in pregnancy associated with use of GDS iron and conventional ferrous sulphate. International Center for Research on Women. Maternal Nutrition and Health Care Program. Research Report Series, Report 5.
18. Brune, M.; Rossander-Hulten, L.; Hallberg, L.; Gleerup, A.; Sandberg, A. (1992). Iron absorption from bread in humans: Inhibiting effects of cereal fiber, phytate and inositol phosphates with different numbers of phosphate groups. J. Nutr. 122: 442 – 449.
19. Hurrell, R.F.; Juillerat, M.A.; reddy, M.B.; Lynch, S.R.; Dassenko, S.A.; Cook, J.D. (1992). Soy protein, phytate and iron absorption in humans. Am. J. Clin. Nutr. 56: 573 – 578.
20. Osagie, A.U. (1998). Anti-nutritional factors In: Nutritional Quality of Plant Foods, Osagie, A.U.; Eka, O.U. (eds.) Ambik Press, Benin City, Nigeria, pp. 221 – 244.