I'm really glad you are thinking about what to recommend to people who are new to chaya. The chemical analyses will be great. But the other thing you might want to include in your article or advice regarding chaya is what can happen when chaya becomes a favored or a crisis food. Quite likely the CN content after whatever preparation is used is not noticed and after even a week or a few months of trial causes no problem. What to WATCH for is goiter problems. **The best place where a lot of research has been done is to look at the cassava literature.** After a quick google search I picked up the following.

1. "I've been reading about foods which suppress thyroid function, and it turns out that tapioca is on the list, amongst other things. Given the extent to which tapioca is used in gluten-free baking, and that Celiac seems to often impact the thyroid, I think the following information is good to know:" https://www.celiac.com/gluten-free/topic/56687-goitrogenic-compounds-in-tapioca-and-other-foods/

2. "Chronic, low-level cyanide exposure is associated with the development of goiter and with tropical ataxic neuropathy, a nerve-damaging disorder that renders a person unsteady and uncoordinated. Severe cyanide poisoning, particularly during famines, is associated with outbreaks of a debilitating, irreversible paralytic disorder called konzo and, in some cases, death. The incidence of konzo and tropical ataxic neuropathy can be as high as three percent in some areas. https://en.wikipedia.org/wiki/Cassava

3. The body is able to detoxify the remaining traces of cyanide during digestion, but as they break down, a goitrogenic compound called thiocyanate is produced. This compound is known to limit the thyroid gland's ability to store and process iodine.
Without iodine, the thyroid is unable to regulate important metabolic processes in adults and trigger key stages of fetal and infant development. Children born to even moderately iodine-deficient mothers risk irreversible brain damage and physical stunting. One study found that the mean IQ of iodine deficient communities was a full 13.5 points lower than that of their iodine-sufficient neighbors.

Goitrogens are so called because by blocking iodine absorption, they encourage the development goiter, the bulbous swelling of the thyroid gland that is the most visible sign of iodine deficiency. Cassava is by no means the only goitrogenic food—lima beans, almonds and bamboo shoots have similar properties, but they are rarely eaten consistently enough or in large enough amounts to pose a health risk, whereas many people eat cassava for several meals a day. And the bulk of cassava consumers live in the world's poorest countries, where iodine deficiency is highest.

https://www.modernghana.com/news/198981/cassavas-link-to-iodine-deficiency-requires-further-study.html

Martin Price, Ph. D.

Here's a lot of information Tim went over with me a while ago that helped me understand what is happening chemically to remove the CN from the leaves of chaya and cassava

"Cassava has a glucoside called linamarin. Some earlier reading I'd done stated that chaya has the same glucoside, linamarin. When cells are crushed (e.g. through chopping/grating/pounding of leaves), it enables the enzyme, linamarase, to come into contact with linamarin to then convert bound cyanide to HCN, the acidic form of CN-.

The link below explains that the enzyme, linamarase, would be deactivated by boiling, so they postulate that cyanide loss is due to leaching as opposed to conversion to HCN and subsequent volitalization of HCN. What happens to "leached" linamarin? Does it stay in the cooking water, in a detoxified state??

A few main points from all this:

--anything one can do to activate the linamarase enzyme helps (e.g., crushing, pounding, chopping of leaves)
--adding water seems to help the process of hydrolysis---but keeping the heat down for a time allows hydrolysis/linamarase to work
--boiling would be helpful from the standpoint of evaporating HCN liberated by linamarase

I had been thinking that boiling would be similar to crushing/pounding as far as rupturing cells and liberating HCN. Apparently, that isn't so, because boiling inactivates linamarase. But, boiling does evaporate HCN liberated by the previous steps (crushing and perhaps low water heat before the boiling point is reached). What I still don't understand, then, is why longer boiling times lead to diminishing HCN being detected by Cyantesmo paper. Maybe, if linamarase denatures at boiling (100C) instead of below boiling (72C), it takes a while until all the linamarase (and hence liberation of HCN) is destroyed."

I hope this helps! And I will get you the formula on Monday at the latest (that when Tim and I meet again)

Stacy Reader
Research & Publication Associate
ECHO Florida

Chronic exposure

Exposure to lower levels of cyanide over a long period (e.g., after use of improperly processed cassava roots as a primary food source in tropical Africa) results in increased blood cyanide levels, which can **result in weakness and a variety of symptoms, including permanent paralysis, nervous lesions (headaches, etc.)**, **hypothyroidism, and miscarriages**. Other effects include mild liver and kidney damage.

On Tue, Aug 29, 2017 at 12:25 PM, Tim Motis <tmotis@echonet.org> wrote:

Hi all. Interesting, Martin, that you mentioned the tech note on low budget research

ideas. I've been working steadily this past week or two on updating that doc. Am adding a few new areas of research while also going back and reviewing what we've learned over the years about research ideas that have been in that doc since a 2010 draft. I noticed that one of the items on that list had to do with quantifying cyanide. In some edits Dawn had done a while back, she alluded to the Bradbury kits that we used to use when I first arrived at ECHO in 2003. Unfortunately, Dr Bradbury retired and I don't see any way to obtain those kits anymore. There are some related papers, though, in case a college/university had chemistry departments willing to try making their own picrate paper and test strips. I'll put this on my "to do" list as far as updating that section with some of the questions that we've been discussing.

I had been scouring the internet for instrumentation for measuring cyanide. Below are two possibilities. I imagine neither are as definitive as chromatography, but they might be worth knowing about:

1) Gas sampling tubes. There are lots of these on the markets. They require a hand-held pump that draws about 100 cc of air through a tube. They are an improvement on the HCN strips we've been using that mainly indicate abence/presence of HCN, but they only measure HCN being vaporized into the air---so you could put chaya leaves in a container of some sort and then insert the tube into the side and sample the gas. There are multiple brands on the market. Here is a website for Gastec tubes:

http://www.zefon.com/store/gastec-hydrogen-cyanide-detector-tubes.html

I know there are pros/cons that influence the accuracy of these tubes; a lot depends on getting a good gas sample, so it is worth getting a quality pump to use with these tubes. One has to consider which brand of gas tubes to use, because often the pumps are made to fit certain tubes.

2) Semi-quantitative test strips.Similar to the Bradbury kit, these allow you to get an idea of cyanide in solutions. Thus, you can get measure cyanide in plant sap (obtained from pressing plant tissue) versus just the HCN liberated as a gas. The same company that we get our HCN strips from also sells slightly more expensive strips that quantify free CN- in solution (or complexes that are decomposed by chlorine). So, from what I gather, they won't tell you how much cyanide is bound up in linamarin, but will at least serve as an indicator of free cyanide in solution. There are links for two products below. I haven't studied them enough to know which is best.
Quantofix Cyanide: http://www.ctlscientific.com/cgi/display.cgi?item_num=91318

Visocolor ECO

Cyanide: http://www.ctlscientific.com/cgi/display.cgi?item_num=931022

One interesting note I read about feeding cyanide-containing foliage of plants like cassava to livestock. Chopping/pounding the leaves before feeding is said to enable the enzyme, linamarase, to break down bound cyanide to HCN that can then volatilize into the air before feeding. Ruminants are more at risk of eating too much raw, unprocessed (e.g. not chopped or pounded beforehand) cassava leaves than monogastrics. The principle is that, as the leaves pass through more digestive steps in ruminant animals, there is a greater chance of bound cyanide being released into their bodies. In monogastrics, on the other hand, some of the bound cyanide at least can pass right through the digestive system and be excreted before it ever gets released as free cyanide into the body. I suppose similar principles apply to chaya, even though cyanide is more concentrated in cassava than chaya, at least with the "bitter" cassava varieties.

Anyway, those were just some thoughts that come to mind. Martin, my goal is to get five or six research topics finalized for the low budget research TN. That will give us something to post on ECHOcommunity. Then, as other topics are updated/developed, we can add to it over time. More so than other TNs, this one will be one that can change over time as new findings and ideas come to light.

Thanks for everyone's insights.

Tim Motis

On Mon, Aug 28, 2017 at 10:02 PM, Martin
Price <mprice.echo@gmail.com> wrote:
Hi all. Interesting, Martin, that you mentioned the tech note on low budget research ideas. I've been working steadily this past week or two on updating that doc. Am adding a few new areas of research while also going back and reviewing what we've learned over the years about research ideas that have been in that doc since a 2010 draft. I noticed that one of the items on that list had to do with quantifying cyanide. In some edits Dawn had done a while back, she alluded to the Bradbury kits that we used to use when I first arrived at ECHO in 2003. Unfortunately, Dr Bradbury retired and I don't see any way to obtain those kits anymore. There are some related papers, though, in case a college/university had chemistry departments willing to try making their own picrate paper and test

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On Aug 29, 2017, at 3:26 PM, Nadezda Amaya <nadezz@hotmail.com> wrote:

On Tue, Aug 29, 2017 at 12:25 PM, Tim Motis <tmotis@echonet.org> wrote:
On Aug 29, 2017, at 2:39 PM, Martin Price <mprice.echo@gmail.com> wrote:

When I stopped working from my ECHO office I brought to my garage boxes of materials that related to subjects that just possibly would be relevant someday. I think some of the correspondence and reports from profs/students were in one of those boxes. I could find these without much trouble if you want to know more detail about any of the subjects.

Martin

This is the 2nd time in a few days on this "forum" where a CN issue has been brought up recently where it would help to have quantitative measurements of CN or cyanogenic compounds (i.e. compounds where CN is part of a more complex molecule and under the right circumstances can release a CN. Let me do some brainstorming with you. Keep in mind that I do not speak for ECHO--this is just

some possible directions you might consider to get the answers you need.

I have a suggestion, but someone at ECHO would need to shepherd the idea. I am assuming it is likely that the quantitative results we need can be done with existing equipment at a college, perhaps a new HPLC column or some such item. You would also need to get a professor to catch the vision who would guide the literature search to evaluate what kind of column is needed and details of the analysis.

For many years ECHO made available an occasionally updated document for Christian colleges called "Low-budget research ideas." I will attach the latest version in case you are interested, though it is no longer updated and this version is at least 5 years old. But the idea sprouted from my own wishes while a biochemistry professor at Geneva College to involve chemistry students in research that would be of practical use but did not require obtaining a big grant to do it. But I didn't know what the questions were.

So early on as I began becoming aware of questions that could be answered and possibly applied by biology or chemistry professors and their students, I wrote the "vision" document--a later version of which is attached.

It was not often used, but there were a few great examples that indeed made a difference. See A Great Example of Research Targeting the Poor. in the attached document if interested.
To the point here, there must be Christian colleges/universities whose science department have equipment used in teaching that could answer these questions. I no longer have a personal network of college professor acquaintances, so cannot point you to many. I also don't have enough experience with the equipment to be of much help. But some colleges come to mind that have close ties with ECHO. E.g. we have 3 interns at the moment who are graduates of Dordt College. So here are a few ideas that one might contact.

Dordt College, one of the few Christian colleges with an agriculture major, did a great job on one of these research ideas, checking whether papaya leaf tea could kill malarial parasites. (It was promising but it needed another student in another year or so to finalize things and that unfortunately did not happen.) Every year a Dordt professor or two bring 5-6 students to our conference.

Indiana Wesleyan has a good science department. One of its biology professors is Dr. Grace Ju, former head of our seed bank, who has led many students in doing

applied research with special attention to Third World needs.

We have also had a lot of interaction over the years with Calvin College in MI. They even invited me as a guest speaker maybe 5 years ago to discuss how their science faculty might get involved. ECHO sends a delegation of one or two students and a regular staff member to their annual Faith and International Development Conference. It is coming up around the first of February.

Margaret Tagwira, wife of Dr. Tagwira who is a professor in the ag department at Africa University in Zimbabwe, is doing some intensive work introducing chaya to that country during the extreme drought they've been facing. She will be speaking at our conference this fall. The college is actively looking to become more and more involved with the small-holder farmers of that country and is probably going to use components of what ECHO calls the Small Farm Resource Center concept in this program. ECHO volunteer tour guide and past VP at Purdue University, John Huie, is very involved there.

Stacy asked about whether we would need to analyze different varieties/accessions. Absolutely. But in addition to differences between varieties, we need to keep in mind that CN probably also varies with the nature of the soil, its pH, and fertility, the intensity of sunlight (e.g. seasonal or grown in shade or full sun), lots of rain or drought, etc. Penny has at least a few varieties here in Florida.

There is also the Institute of Nutrition of Central America and Panama that has at times been very involved and gave initial guidance to Penny. EARTH University in Costa Rica is another possibility.

Martin (see attached)