Virtual meeting on Teams

Nov 30, 2023 from 6-8 pm SGT

AOAC Virtual work session on the needs for standard development to measure cyanide in cassava, cassava products and other food materials

A collaboration between the Harmonization of Methods Working Group of AOAC SEA and AOAC INTERNATIONAL

Moderator: Erik Konings, Ph.D.

How the project got started

AOAC INTERNATIONAL

European Union Reference Laboratory

WAGENINGEN UNIVERSITY & RESEARCH

Nestlé

ARSO

African Organisation for Standardisation

DOST-ITDI, PH

AOAC INTERNATIONAL SOUTHEAST ASIA SECTION

APFAN Asia Pacific Food Analysis Network

Australian Government Department of Industry, Science and Resources

National Measurement Institute

The topic was first introduced at AOAC SEA's Harmonization of Methods Working Group

Key people in the project

Annabelle Briones, Ph.D.
DOST-ITDI, The Philippines, Director
AOAC SEA HoMWG Chair

Stewart Jones
Asia Pacific Food Analysis Network (APFAN), Coordinator
AOAC SEA CBWG Co-chair

Deni Taleski, Ph.D.
National Measurement Institute, Australia, Technical Development Officer

Patrick Mulder, Ph.D.
Wageningen Food Safety Research (WFSR), The Netherlands; EURL on Mycotoxins & Plant Toxins, Research Scientist

Xinping Hou, Ph.D.
BV-AQ, Manager
AOAC SEA President Chair

Tetsuhisa Goto, Ph.D.
Central Science Commerce Inc, Japan, Technical Advisor
AOACI member since 1983; Chair for Method Committee D, 2004-2007

Emmanuel Kirwa
African Organisation for Standardisation (ARSO)

Kate Mastovska, Ph.D.
AOAC INTERNATIONAL, Deputy Executive Director & Chief Science Officer

Erik Konings, Ph.D.
Nestlé Institute of Food Safety and Analytical Sciences, Expert
AOAC SEA Mentor
AOACI Past President

Cyanide in cassava is a Food Safety Issue

Cassava is the staple food of about 600 million people in the Tropics but produces natural cyanogens and cyanogenic glycosides.

- '**Konzo**' is a permanent paralysis of the legs caused by cyanogen exposure particularly in children.
- **Konzo** happens quickly and is incurable

Cyanide in cassava is a Food Trade Issue

- Recent discrepancies have been determined between the *acid hydrolysis method**, and an *enzymatic hydrolysis* test kit developed by the Australian National University (ANU)**.
- **Is cyanide content underestimated by the traditional acid hydrolysis method?**
- In 2022/3, the National Measurement Institute Australia (NMIA) developed an (National Association of Testing Authorities-NATA) accredited method for enzymatic digestion.

** **acid hydrolysis** method relies on acid digestion and trapping of the generated hydrogen cyanide in alkaline solution, followed by derivatisation and colourimetry for determining concentration.*

*** **enzymatic digestion** method utilises enzymes which facilitate release of hydrogen cyanide from cyanogenic glycosides e.g. linamarin. Hydrogen cyanide migrates to an alkaline trapping solution in a closed system followed by derivatisation and colourimetry for determining concentration.*

FSANZ regulatory limits

Australia New Zealand Food Standards Code - Schedule 19 - Maximum levels of contaminants and natural toxicants

S19-6 Maximum levels of natural toxicants

(1) For each natural toxicant listed below, the maximum level (in mg/kg) for a particular food is listed in relation to that food:

	Maximum levels of natural toxicants	
Hydrocyanic acid, total	Confectionery	25
	Stone fruit juice	5
	Marzipan	50
	Ready-to-eat cassava chips	10
	Alcoholic beverages	1 mg per 1% alcohol content

- ***Hydrocyanic acid, total*** *means all hydrocyanic acid including hydrocyanic acid evolves from cyanogenic glycosides and cyanohydrins during or following enzyme hydrolysis or acid hydrolysis.*
- ***Ready-to-eat cassava chips*** *means the product made from sweet cassava that is represented as ready for immediate consumption with no further preparation required, and includes crisps, crackers and 'vege' crackers.*

Regulatory continued

Commission Regulation (EU) 2022/1364 amending Regulation (EC) No 1881/2006 as regards maximum levels of hydrocyanic acid in certain foodstuffs.

Foodstuffs([1])		Maximum level (mg/kg)
'8.3	**Hydrocyanic acid, including hydrocyanic acid bound in cyanogenic glycosides**	
8.3.1	Unprocessed whole ([60]), ground, milled, cracked, chopped linseed with the exception of foodstuffs listed in 8.3.2 ([54])	250
8.3.2	Unprocessed whole, ground, milled, cracked, chopped linseed placed on the market for the final consumer ([54]) ([55]) (*)	150
8.3.3	Unprocessed whole, ground, milled, cracked, chopped almonds placed on the market for the final consumer ([54]) ([55]) (*)	35
8.3.4	Unprocessed whole, ground, milled, cracked, chopped apricot kernels placed on the market for the final consumer ([54]) ([55])	20
8.3.5	Cassava root (fresh, peeled)	50
8.3.6	Cassava flour and tapioca flour	10

Hydrocyanic acid *is a highly toxic substance. While it is not present in food at toxicologically relevant levels, it is released when plant-derived foods containing cyanogenic glycosides are chewed or otherwise processed and those glycosides enter into contact with hydrolytic enzymes. As hydrocyanic acid always forms as a mixture of nondissociated acid and dissociated cyanide ions, the health-based guidance value is calculated for this mixture, referred to as 'cyanide'.*

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Regulatory continued

CODEX ALIMENTARIUS International Food Standards

- 4 Codex standards:
  - Standard for sweet cassava (CXS 238-2003)
    - In the absence of a Codex maximum level for hydrogen cyanide, an acceptable maximum level shall be set on a safety basis by the national legislation of the importing country.
  - Standard for edible cassava fluor (CXS 176-1989)
    - Methods recommended in CSX 234-1999 shall be used to check compliance with standard.
  - Standard for bitter cassava (CXS 300-2010)
    - Bitter varieties of cassava are those containing more than 50 mg/kg of cyanides expressed as hydrogen cyanide (fresh weight basis).
    - In the absence of a Codex maximum level for hydrogen cyanide, an acceptable maximum level shall be set on a safety basis by the national legislation of the importing country.
  - Standard for fermented cooked cassava-based products (CXS 334R-2020)
    - The values of total hydrocyanic acid will be determined subject to the completion of the ongoing work in the Codex Committee on Contaminants in Food (CCCF).
    - Methods of Analysis: ‘to be developed’
- No method for cyanide recommended in CXS 234-1999

Regulatory continued

CODEX ALIMENTARIUS International Food Standards

General Standard for Contaminants and Toxins in Food and Feed CXS-193-1995

CXS 193-1995
HYDROCYANIC ACID

Reference to JECFA:	39 (1992), 74 (2011)
Toxicological guidance value:	ARfD 0.09 mg/kg bw as cyanide (2011, this cyanide equivalent ARfD applies only to foods containing cyanogenic glycosides as the main source of cyanide) PMTDI 0.02 mg/kg bw as cyanide (2011)
Contaminant definition:	See explanatory notes in the column “Notes/Remarks”
Synonyms:	HCN
Related code of practice:	Code of Practice for the Reduction of Hydrocyanic Acid (HCN) in Cassava and Cassava Products (CXC 73-2013)

Commodity/ Product name	Maximum level (ML) mg/kg	Portion of the commodity/ Product to which the ML applies	Notes/Remarks
Gari	2	Whole commodity	The ML is expressed as free hydrocyanic acid. Relevant Codex commodity standard is CXS 151-1989.
Cassava flour	10		The ML is expressed as total hydrocyanic acid Relevant Codex commodity standard is CXS 176-1989.

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Regulatory continued

EAST AFRICAN STANDARD

EAS 739:2010 Dried cassava chips - Specification

Table 1 - Compositional requirements for dried cassava chips

Parameter	Requirement	Method of test
Moisture content, by mass, %, maximum	12	ISO 712
Crude ash on dry matter basis %, maximum	3	ISO 2171
Acid insoluble ash, on dry matter basis, %, maximum	0.15	EAS 82
Crude fibre on dry matter basis %, maximum	2	ISO 5498
Hydrogen cyanide mg/kg, dry matter basis max	10	DEAS 744

***DEAS 744**, Cassava and cassava products — Determination of total cyanogens — Enzymatic assay method*

AOAC Official Methods

	Hydrocyanic acid	Cyanogenetic glucosides	
AOAC 920.144	In almond extract		Titration
AOAC 915.03	In beans		Autolysis in water, titration
AOAC 970.11	Aminal feed		Autolysis in water, titration
AOAC 936.11		Animal feed	Qualitative test

Challenges / Gaps in cyanide analysis

What are the issues?

- Cyanide levels in food (i.e. cassava, cassava products, almonds, beans, linseed, apricot kernels) are globally regulated.
- Current standard methods use either **acid hydrolysis** or **enzymatic cleavage** to arrive at a value for hydrogen cyanide in these materials, but these methods may produce different results.
- Standard Methods, Reference Materials, Proficiency Testing studies / interlaboratory studies are not available.

What do we need to do?

- Need to develop two Standard Method Performance Requirements for quick, cheap, field-based screening (analogous to Identification Tests in pharmacopoeias) and rigorous, quantitative/confirmatory, in-the-lab testing (analogous to Assays in pharmacopoeias)
- Need to organize collaborative studies to demonstrate that a method performs well compared to requirements.
- Need Experts for Standard Development and Expert Review Panel
- Need to find fundings for the project

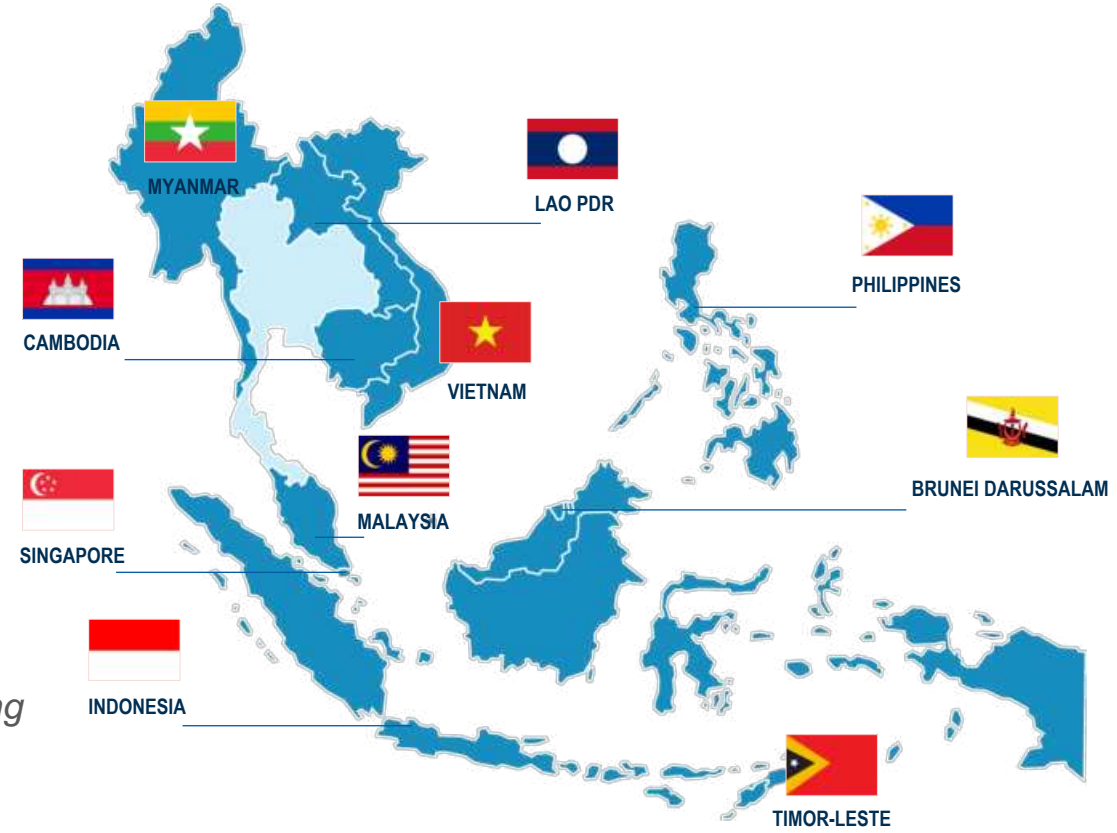
AOAC INTERNATIONAL and its 19 Sections

China
India
Japan
Southeast Asia
Taiwan
Thailand
Europe
Lowlands
Latin America-Caribbean
Arab Section
Sub-Saharan Africa
Central US
Mid-Atlantic US
Mid-Canada
Midwest US
Pacific NW US
Pacific SW US
Southern US
Southern California US

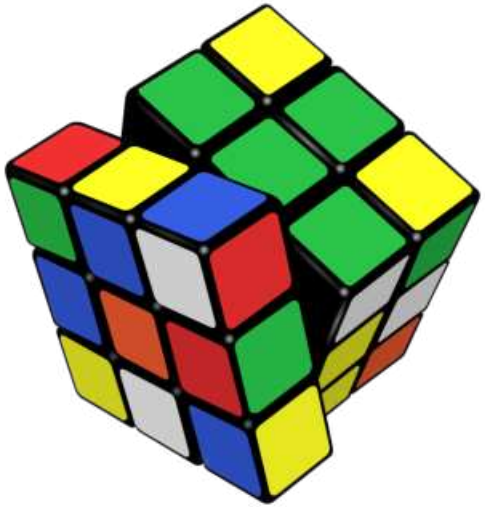
AOAC Southeast Asia Section

- Brunei
- Cambodia
- Indonesia
- Laos
- Malaysia
- Myanmar
- Philippines
- Singapore
- Timor-Leste
- Vietnam

** AOAC Thailand Section is merging into AOAC SEA Section*



Five Working Groups



Capacity Building Working Group

To develop competences, capacity and analytical capabilities for implementation of key processes of detection, assessment, response, notification, and monitoring of events

Emerging Issues Working Group

To identify and monitor emerging issues to forecast and predict possible food safety risks, and develop appropriate measures to manage the identified risks

Harmonization of Methods Working Group

To identify methods to harmonize in line with SOUTHEAST ASIA priorities in order to achieve uniformity of results, or conversion of results such that different countries can use the data obtained from different laboratories

Microbiology Working Group

To identify needs associated with the implementation of microbiological food safety by driving scientific agreement leading to a reduction in foodborne microbial incidents and recall along with microbiological capability development

Training of Young Scientists Working Group

To establish programs across Southeast Asia countries to develop, train, encourage, support, and recognize young scientists

AOAC INTERNATIONAL SOUTHEAST ASIA SECTION

Expanding network of AOAC SEA Organizational Members

AOAC INTERNATIONAL SOUTHEAST ASIA SECTION

Tireless giving back to the community

Student Winner	Program	University	Country
Nol, Rathana	Undergraduate	Royal University of Agriculture	Cambodia
Bion, Abigail Grace	Graduate	University of Santo Tomas	Philippines
Sujana, Ratih Dewi	Graduate	IPB University	Indonesia
Chanthavong, Oudone	Undergraduate	National University of Laos	Laos
Tan, Hui Ru	Graduate	National University of Singapore	Singapore
Wei En, Chua	Undergraduate	Republic Polytechnic	Singapore
Tran, My	Undergraduate	VNU University of Science	Vietnam
Nguyen Dinh, Thi	Undergraduate	VNU University of Science	Vietnam
Kieu, Anh	Undergraduate	VNU University of Science	Vietnam
Mohammad Yunus, Faizah	Graduate	University of Malaya	Malaysia
Mohamed, Ahmad Husaini	Graduate	Universiti Sains Malaysia	Malaysia
Abd Hamid, Muhammad Ariffuddin	Graduate	Universiti Sains Malaysia	Malaysia

Facilitate regulation/standards harmonization for food safety

- Local and global regulators, industry, and academia have worked together to harmonize their regulations in order to simplify the exchange & trading between countries and ensure food safety and compliance
- Southeast Asia countries play important roles in global food production, supply and trading
- AOAC INTERNATIONAL, AOAC SEA and other regional Sections can play an important role to facilitate regulation/standards harmonization in terms of food safety
- **Cyanide testing in cassava, cassava products, and other materials is a technical issue that can be solved with AOAC's method development process and implemented by its network of users and partners**

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