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DISCUSSION PAPER ON THE ESTABLISHMENT OF MLS FOR HCN IN CASSAVA AND CASSAVA-BASED PRODUCTS AND OCCURRENCE OF MYCOTOXINS IN THESE PRODUCTS

(Report of the EWG - updated by Nigeria as chair of the EWG)

BACKGROUND

1. At CCCF11 (2017), the FAO/WHO Coordinating Committee for Africa (CCAFRICA) requested to know if it is appropriate to extend the existing ML for HCN of 2 mg/kg in gari to fermented cassava products, and whether mycotoxins were of public health concern in these products.
2. Based on the requests of CCAFRICA, CCCF11 recommended[1] an EWG to be chaired by Nigeria be established to prepare a discussion paper:
    a. On the need and feasibility to establish an ML for HCN in all fermented cassava products and address the issue of harmonizing the expression of HCN levels, i.e. free or total HCN.
    b. Source for data on mycotoxins occurrence in these products that will allow CCCF determine whether mycotoxin contamination would be of public health issue in these products.
3. The EWG carried out its mandate and submitted the Discussion Paper, CX/CF 18/12/13 for consideration by CCCF12, but it could not be discussed due to inadvertent absence of Nigeria, the EWG Chair. The discussion paper was defered for presentation at CCC13 while members were encouraged to continue submitting new data on GEMS/Food platform.[2]
4. The Discussion Paper (CX/CF 18/12/13) has been updated (*with updates reflected in italics*) withthe following conclusions and recommendations for consideration by CCCF13. Information and data in support of the recommendations are presented in Appendix I.

CONCLUSIONS AND RECOMMENDATIONS-

5. The improving global profile of cassava as raw material for human foods, animal feeds, pharmaceutical and confectionary industries has been presented in this discussion paper. Its obvious growing significance in export trade is also highlighted. The health impact of the antinutritional factors in cassava and cassava based products namely hydrocyanic acid and mycotoxins was discussed. These were all done to advise CCCF 12 on the feasibility of setting MLs for HCN in cassava fermented products and elucidate whether mycotoxin contamination is of public health concern. These efforts led to the following inferences and recommendations:

(a) The levels of HCN found in three West African cassava fermented food products; *gari, lafun* and *fufu* were above the ML of 2.00 mg/kg free hydrocyanic acid set for gari, which implies that most of them may result in intakes above the recommended PMTDI of 0.02mg/kg body weight.

(b) The discussion paper reveals the presence of aflatoxins, ochratoxinA, deoxynivalenol, 15-acetyl deoxynivalenol, fumonisin B_1 and B_2, zearalenone, α-zearalenol and fusarenon-X in gari, lafun, cassava chips, cassava flour and bread from six African countries namely Nigeria, Cameroon, Uganda, Kenya, Sierra Leone and Rwanda.

(c) About 20 of the 581 cassava food products tested from the six countries had total aflatoxin levels above the limit of 10 µg/kg and only 3 of 28 sample analyzed for aflatoxin B_1 from Nigeria had toxin content above the EU regulated limit of 2µg/kg. However, 10 of the 18 samples of gari analyzed for ochratoxin A also from Nigeria contained the toxin above the regulated limit of 5µg/kg. Except for these toxins, aflatoxins and ochratoxin A, all other toxins occurred at concentrations below the limits chosen

[1] REP17/CF paras 14-15

[2] REP18/CF, para. 125

for the purpose of drawing inferences for discussion.

(d) If the data in "5c" above are considered enough to draw inferences, aflatoxins and ochratoxins would be the only toxins of public health concern in *gari.*

(e) However, the skewness of the data involved in this work to only Africa and the limited data (18 samples) on ochratoxin A used, would not allow the inferences (5b-d) drawn from the work to be representative of the world.

(f) In view of "5e" above and the one year grace granted, further data and information were sourced on HCN and mycotoxins in cassava based products to reflect global representation especially from Africa, Asia, Southern and Central Americas and Caribbean.

(g) *Within the year under review, four studies that considered mycotoxins in cassava and cassava products are being reported, three of which used LC-MS/MS method for quantification while one used ELISA. In the first study, 23 mycotoxins were assayed in 41 gari samples from 5 agroecological zones of Nigeria, 9 of these mycotoxins are currently regulated by EU and some other countries in ready-to-consume foods. DON and NIV were not detected in any of the samples, aflatoxins and ochratoxins previously considered as a problem appeared at very low concentrations.*

(h) *The second study, a total diet study, surveyed 12 composite dried cassava and 8 composite fresh cassava samples homogenised from 144 and 96 individual samples respectively, within four African countries (Benin, Cameroon, Mali and Nigeria) in 2018 for mycotoxins and other metabolites. Dried cassava was seen to have the presence of most of the important mycotoxins while fresh cassava had only ochratoxin A among the major mycotoxins. The study revealed that the incidence and levels of most studied mycotoxins were low; 1/10 aflatoxin B1, 1/10 ochratoxin A, 3/13 FB2, 2/12 zearalenone among others, while fresh samples had 1/8 ochratoxin A. In a third study from Kogi North-Central Nigeria ELISA was used to assay total aflatoxin, fumonisins and ochratoxins. Precisely, eight of ten (8/10) gari samples, 9/10 fufu samples and 10/10 cassava flour samples contained aflatoxin, 3 of the gari samples were contaminated beyond 4ppb. Ochratoxin was present in 1 gari, 5 fufu, and 2 flour samples respectively, gari contamination was above 3ppb. Fumonisin was present in 3 gari and 4 fufu samples at very low concentrations and not detected in flour samples.The fourth study which analysed imported samples of cooked and raw cassava in USA reported no mycotoxin in 60 samples.*

(i) *In three reports, cassava and cassava products from different parts of Nigeria were subjected to HCN analyses while those from Benin, Mali, Cameroon and Nigeria were analysed for cyanogen glycosides. Sixty (60) of the sixty one (61) gari samples analysed were contaminated with HCN, of which 13 had values less than 1.0 mg/kg, 36 had values above 1.0 mg/kg but below 1.5 mg/kg, 10 had values above 1.5 mg/kg but below 2.0 mg/kg, and 1 sample had value above 2mg/kg. Another study considered the levels of linamarin and lotaustralin. Linamarin is enzymatically cleaved by linamarase to release HCN. Incidence of linamarin in fresh cassava samples was 6/8 while that of dried cassava was 12/12 both from composite sampling mentioned in (h). Proportionally levels were higher in fresh cassava than dried cassava. This is indicative that drying of cassava before further processing will reduce the resulting HCN levels and confirming the effectiveness of best practices advocated in COP for Reduction of HCN in Cassava and Cassava Products (CAC/RCP 73-2013). In another study considering 10 samples of gari from Kogi, North-Central Nigeria, 5 were positive, 1 was above 1.5 mg/kg but below 2.0 mg/kg. Ten (10) samples of fufu had no HCN. Cassava flour, which is an unfermented product was also analysed and 9/10 had HCN of which 5 were above 2.0 mg/kg. This is a clear indication of the role of fermentation and processing in reduction of cyanide level.*

(j) *HCN levels appeared at high incidence in fermented cassava products but at low levels. Further processing as in the case of fufu has shown to reduce HCN to the barest minimum or remove it. Unfermented cassava flour pose as a bigger threat for HCN toxicity from cassava especially because it does not require further processing beyond warm water mixture before its consumption; processing do not involve decantation of water.*

(k) *A total of 49 mycotoxins including the major and emerging mycotoxins were analysed in fermented cassava product. From 423 samples HCN had 60.8% (257) incidence with 40.2% (170) being above 2mg/kg. Exactly 29.3% of 375 samples were contaminated with AFT, 18% of 22 samples were contaminated with AFB1,24.8%of 133 samples had FB1, 37.4% of 91 samples had OTA, 18.8% of 101 samples had DON, 33.6% of 149 samples had ZEA, 12.5% of 24 samples were positive for T2-Toxin. While most of the reported mycotoxins remained at lower levels below safe limit, 4.92%, 17.72% of AFT, OTA respectively had values that are considered unsafe by regulations. Joint occurrence of mycotoxins in samples could have synergistic, potentiative, antagonistic or additive effects which is a cause for public health concern. Longer duration of fermentation and changing of the steep water have proven to be effective in reducing mycotoxins and HCN levels.*

(l) *On whether it is appropriate to extend the existing ML for HCN of 2 mg/kg in gari to fermented cassava products, we make the following submissions:*

i. Data of HCN in a total of 443 samples of cassava food products were used in this work, 348 of the samples were gari, 75 of Fufu and 10 each of cassava flour and fresh unfermented cassava (refer to Table 4.0). While 192 of the gari samples were contaminated by HCN, 104 (29.9%) of these samples have HCN levels above the regulated limit of 2.0mg/kg. Sixty five (86.7%) of the fufu samples had HCN above the safe limit. Although 9 of the cassava flour samples were contaminated, 6 of the samples had HCN levels above regulated limit. However, none of the fresh unfermented cassava samples were contaminated with HCN.

ii. Based on '(l)i' above, if the existing ML for HCN in gari is to be extended to fermented cassava products, it should now be to fufu which has about 87% HCN contamination above legislated level of 2.0 mg/kg. While more data are required to take decisions on other fermented products.

iii. The data on 10 samples of cassava flour is too small a sample size to make valid conclusions.

(m) *With regards to whether mycotoxins were of public health concern in fermented cassava products, the EWG hereby makes the following submissions*

i. Forty nine fungal metabolites (Table 4.1) were analyzed for in varying numbers of samples of gari (414), fufu (10), lafun (36), chips (164), flour (33), fresh/unfermented (8), cooked (40) and raw (20) cassava.

ii. Although gari was contaminated by 45 mycotoxins, only total aflatoxin, ochratoxin and zearalenone were found at concentrations above their respective MLs. One percent of 414 samples, 39.3% of 28 samples and 1% of 53 samples of gari had AFT, OTA and ZEN levels respectively above legislated limits. FUS-X, Methylsulochrin, integracin A and B, and Methylfunicone were not present in the tested gari samples. It is pertinent to also mention that there was multi-occurrence of major, minor and emerging mycotoxins in the tested product samples.

iii. Total aflatoxin and fumonisins were detected in fufu at levels below ML but ochratoxins were found in 5 of the 10 tested samples and 3 of the contaminated samples had ochratoxin content above ML.

iv. Fumonisin B1 and B2, zearalenone, α-zearalenone, DON, 3-acetyldeoxynivalenol, DAS and FUS-X were detected in Lafun but were all present at concentrations below safe limits.

v. Nineteen of the 164 cassava chip samples tested had AFT at above safe limits

vi. AFT and ochratoxin were found in cassava flour however, only 3 of the 10 samples had AFT above the regulated limit

vii. Ochratoxin A was found in 1 of 8 tested samples but at level below safe limit.

viii. AFB1, AFB2, AFG1, AFG2, FB1, FB2, FB3, OTA, T-2, ZEN and DON were tested for but not found in all the 40 cooked and 20 raw cassava samples. Based on a-h above, Total Aflatoxin and ochratoxins are the mycotoxins of public health concern in both fermented and unfermented cassava products. The presence of ZEN in 1 out of 53 gari samples at above regulated limit (b above) is not sufficient evidence to classify it as a mycotoxin of public health concern in fermented cassava product. The simultaneous occurrence of major, minor and emerging mycotoxins in cassava products as observed in this work which could have synergistic and additive adverse human health effects necessitates a holistic approach in the prevention and reduction of mycotoxins rather than focusing only on those with public health concern.

APPENDIX I

BACKGROUND INFORMATION
(For information to Codex Members and Observers when considering the conclusions and recommendations)

Consideration of MLs for HCN in CODEX

1. The 30thSession of the Codex Alimentarius Commission (CAC30) (2007) concurred with the recommendation of the 59thSession of the Executive Committee (CCEXEC59) (2007) to adopt the proposed draft Standard for Bitter Cassava, as elaborated by the Committee on Fresh Fruits and Vegetables (CCFFV) at Step 5 and that, as a separate issue, the Committee on Contaminants in Foods (CCCF) should consider the safety levels of hydrogen cyanide (HCN) proposed in the Standard, with a view to a re-evaluation of cyanogenic glycosides (CG) by the Joint FAO/WHO Expert Committee on Food Additives (JECFA).[3]
2. CAC31 (2008) sent the draft Standard for Bitter Cassava back to CCFFV for further work (step 6) on the labelling and processing of bitter cassava due to the recognized safety concerns if cassava is consumed without adequate processing, with a view to referral to the Committee on Food Labelling (CCFL) for re-consideration.[4]
3. The proposed levels for HCN of the Draft Standard for Bitter Cassava are as follows *in italic*: *[Bitter varieties of cassava are those that contain more than 50 mg/kg but less than 200 mg/kg HCN (fresh weight basis). In any case, cassava must be peeled and fully cooked before being consumed.]*
4. CCCF02 (2008) considered the need for a re-evaluation of cyanogenic glycosides by JECFA.
5. Pivotal to considerations on the safety of bitter and sweet cassava is whether the current preparation instructions are adequate to ensure safe consumption of these foods. It is unclear what level of processing is required for different initial levels of cyanogenic glycosides in bitter cassava. For example, it is not clear to what extent, following peeling and cooking, additional preparation techniques are necessary to adequately reduce the risk for cassava with 50 mg/kg HCN (fresh weight basis) compared with 200 mg/kg HCN (fresh weight basis).
6. The same CCCF noted that potential excessive dietary exposure to cyanogenic glycosides, mainly from cassava but also from other products, was assessed at the 39th Meeting of JECFA (JECFA39) (1992) and that, due to lack of quantitative toxicological and epidemiological information at that time, JECFA could not conclude on a safe level of dietary exposure for this naturally occurring toxicant. However, JECFA (WHO 1993) had also concluded that a level of up to 10 mg/kg HCN in the *Standard for Edible Cassava Flour* (CXS 176-1989) was not associated with acute toxicity. A review of the available data by the European Food Safety Authority in 2004 reached a similar conclusion.
7. There are a few FAO publications addressing good agricultural and manufacturing practices for the growing and processing of cassava, including other ongoing work in this field, to assist countries with the cultivation, processing and handling of this product. This information should be taken into account if a Code of Practice or an ML is considered necessary for cyanogenic glycosides in the future.
8. The CCCF02 agreed[5] that an electronic working group (EWG), led by Australia, prepare a discussion paper which should include an overview of available data on cyanogenic glycosides with a view to possible re-evaluation by JECFA. The EWG in its submission made the following recommendations:
    a. JECFA is requested to re-consider the data available on cyanogenic glycosides and advise on the public health implications of cyanogenic glycosides and their derivatives in food. In particular, whether there are sufficient data to establish an appropriate health standard, such as an acute reference dose or tolerable daily limit, for cyanogenic glycosides or their derivatives present in food.
    b. JECFA to consider whether or not the current level of up to 10mg/kg HCN in the *Standard for Edible Cassava Flour* is still an appropriate level which is not associated with acute toxicity, and whether this level would be applicable to any other HCN-containing food.
    c. JECFA to consider what levels of these cyanogenic glycosides and their derivatives may be appropriate in food, including levels that are appropriate to minimise any risks to public health from the consumption of foods containing cyanogenic glycosides and their derivatives.
    d. JECFA to consider what an appropriate descriptor for total HCN in food could be.

[3] ALINORM 07/30/REP para 92
[4] ALINORM 08/31/REP paras 37-39
[5] ALINORM 08/31/41 para 180

e. Taking into account any assessment by JECFA, that CCCF consider developing a Code of practice for producing, processing and marketing of foods which may contain cyanogenic glycosides or their derivatives. In consultation with CCFL, this would also include whether further information requirements are necessary for these foods to ensure adequate processing of cyanogenic glycoside-containing foods by consumers before consumption.

f. Following receipt of any risk assessment advice from JECFA, CCCF and the Committee on Methods of Analysis and Sampling (CCMAS) should review the current relevant Codex Standards to ensure these standards are consistent in relation to any limit for cyanogenic glycosides and their derivatives in food.

9. At CCCF03 (2009), Australia presented a discussion paper on cyanogenic glycosides. The CCCF agreed[6] to request JECFA to re-consider the data available on cyanogenic glycosides and advice on the public health implications of cyanogenic glycosides and their derivatives in food. In addition, and taking into account any assessment by JECFA, the CCCF would consider developing a code of practice for producing, processing and marketing of foods which may contain cyanogenic glycosides or their derivatives.

10. JECFA72 (2010) JECFA conducted a risk assessment of cyanogenic glycosides in foods. Cyanogenic glycosides can cause acute poisoning in humans as well as several chronic diseases associated with under-processed cassava production. JECFA established health-based guidance values (HBGVs) for cyanogenic glycosides; namely, an Acute Reference Dose (ARfD) of 0.09 mg/kg body weight, expressed as cyanide equivalents and a Provisional Maximum Tolerable Daily Intake (PMTDI) of 0.02 mg/kg body weight, as cyanide.

11. Estimates of dietary exposure used conservative estimates (total conversion of cyanogenic glycosides to hydrogen cyanide and without taking account of effects of food preparation or processing in most cases). They indicate possible exceedances of the acute and sub-chronic reference doses in some population groups.

12. Given these possible health impacts, it is important to consider whether existing MLs in commodity standards are protective and whether MLs in other commodities are warranted. It is also appropriate to develop guidance to reduce the concentrations of HCN in foods.

13. CCCF06 (2012) agreed to establish an electronic working group led by Australia and co-chaired by Nigeria to start new work on a code of practice and maximum levels for hydrocyanic acid in cassava and cassava products for comments at Step 3 and consideration by the next session.[7]

14. At CCCF07 (2013), the EWG which worked on the review of the MLs, development of a code of practice (COP) and identifying of analytical methods suitable for analysis of HCN in foods made the following recommendations that were adopted:[8]

    a. Revision or establishment of new MLs for HCN in cassava and cassava products

        i. It is recommended that a common approach is used for expressing MLs relating to HCN generated from naturally occurring cyanogenic glycosides. The EWG recommends that total HCN should refer to all cyanogenic glycosides, cyanohydrins and “free” HCN in a food as described in the most recent JECFA evaluation of 2012.

        This would require amending the ML for gari to express it in terms of total HCN, rather than free hydrocyanic acid. It is recommended that the ML for gari be converted to a value reflecting the total HCN level. Since JECFA *was not able to characterize the risk from consuming gari, this conversion could be based on the current level, pending generation of further consumption and occurrence data. The CCCF should consider whether new work is proposed on the descriptor or whether this should be deferred until reconsideration of MLs for other cassava products at a later date.

        ii. Currently there are no MLs for HCN in cassava in the *General Standard for Contaminants and Toxins in Food and Feed* (CXS 193-1995) (GSCTFF). Instead the types of cassava (bitter or sweet) are distinguished by an HCN concentration of 50 mg/kg in their respective standards. It may be appropriate to incorporate MLs for HCN derived from cyanogenic glycosides into the GSCTFF at some point. However it would be more appropriate to make this decision once further information is available to fill the current data gaps.

[6] ALINORM 09/32/41 para 108
[7] REP12/CF paras 165-168
[8] REP13/CF paras 89-92

iii. In the absence of a Codex ML for hydrogen cyanide for bitter cassava in the GSCTFF the standard for bitter cassava permits the setting of an acceptable maximum level on a safety basis by the national legislation of the importing country pending the outcome of the work of the Committee on Contaminants in Foods on cyanogenic glycosides. It is recommended that this approach is retained until further information is available on the effects of processing and levels in final products derived from bitter cassava.

iv. For cassava flour, there are no available estimates of dietary exposure that exceed the ARfD or PMTDI, and therefore there is no need to amend the current ML.

v. For other cassava products at this time new MLs should not be developed because of the conservatism and uncertainty of the risk assessment and the need for further information on concentrations of HCN in cassava-based foods.

vi. Other risk management strategies, particularly the development and implementation of a COP, should be prioritized. Further data should be collected after the code of practice is in place and its effectiveness should be evaluated before consideration is given to setting new MLs. This should be accompanied by other education and outreach initiatives.

vii. Countries should be encouraged to continue to collect data on concentrations of total HCN in cassava and cassava based products, methods of preparation and consumption amounts after implementation of the code of practice. Data are needed on how much cassava and cassava products are consumed and what concentrations of HCN are in the different cassava products eaten in different regions.

b. Methods of analysis

i. A variety of fit-for-purpose analytical methods may be used to determine occurrence levels for total HCN in cassava and its products.

ii. Further validation work is required for the analytical methods used to measure total HCN.

c. Development of a COP

i. Appropriate code of practices for effective reduction of HCN in the following processed cassava products: gari, fufu and fufu powder, dried cassava chips and other cassava products: Lafun, Atteke, Chikwangue, Bila, Farinha, Bikedi and NtobaMbodi; were recommended.

ii. Recommended practices based on GAP and GMP for reduction in HCN in various cassava products were also given.

15. Current Codex and international standards and texts

a. CAC has developed and published standards for sweet cassava, bitter cassava, edible cassava flour and gari (a product obtained from processing cassava tubers) (also spelt as “garri”). The key aspects of these standards are:

i. Sweet cassava is defined as a raw product containing less than 50 mg/kg of “hydrocyanic acid”.

ii. Edible cassava flour is defined as a product suitable for direct human consumption and the level of “total hydrocyanic acid” in the flour must not exceed 10 mg/kg.

iii. For gari, another product for direct human consumption, the “total hydrocyanic acid” must not exceed 2 mg/kg as free hydrocyanic acid.

iv. The standard for bitter cassava (300-2010) defines bitter varieties of cassava as those containing more than 50 mg/kg of cyanides expressed as hydrogen cyanide (fresh weight basis).

v. In the absence of a Codex maximum level for hydrogen cyanide for bitter cassava in the GSCTFF it permits the setting of an acceptable maximum level on a safety basis by the national legislation of the importing country pending the outcome of the work of the Committee on Contaminants in Foods on cyanogenic glycosides.

b. Labelling provisions in the standard for sweet cassava require a statement that cassava must be peeled and fully cooked before being consumed.

c. The labelling requirements for bitter cassava to alert consumers to risk of consumption are:

i. cassava must not be eaten raw

ii. cassava shall be peeled, de-pithed, cut into pieces, rinsed and fully cooked before consumption

iii. cooking or rinsing water must not be consumed or used for other food preparation purposes.

d. CAC36 (2013), based on recommendations from CCCF07, adopted a Code of Practice for the Reduction of Hydrocyanic Acid in Cassava and Cassava Products, aimed at assisting primary producers and processors on best practices which will ensure elimination or reduction to the lowest possible level of Hydrocyanic acid in cassava.[9]

e. MLs for total HCN have been established in a few countries for cassava and cassava derived foods including ready-to-eat cassava chips/crisps and these are for a limited range of substances (**Annex 1)**

General Information on Cassava and Fermented Cassava Products

16. Cassava (*Manihotesculanta*Crantz) is a shrub with tuber root containing high starch which belongs to the Euphorbiaceae family. The shrub which is mainly grown for its starch was domesticated in West-Central Brazil by 4600 BC (Pope *et al*. 2001) and was introduced to Africa from Brazil in the 16th Century by Portuguese traders. There are many cultivars and species of cassava but are all classified into bitter and sweet varieties according to the cyanogenicglucoside contents of their roots. The bitter and sweet varieties have high (≥ 100/mg/kg) and low (≤ 50 mg/kg) HCN content respectively.

17. It is the sixth most produced and consumed crop in the world after sugar cane, maize, rice, wheat and potatoes (FAOSTAT, 2017). It is cultivated in 103 countries covering a total of 23, 482.052 hectares of land in the world. World production ofcassava are from40 African, 12 South American, 16 Asian and 13 Oceania countries with Africa producing more than 50% of the crop. The ten biggest producers of cassava in 2016, in decreasing order, are Nigeria, Thailand, Brazil, Indonesia, Ghana, Democratic Republic of Congo, Vietnam, Cambodia, Angola and Mozambique.According to FAOSTAT (2014), the world net production value of cassava in 2014 was $26.1 billion US dollars.

18. The tuber root plant is drought resistance and therefore thrives and is grown in arid soil of tropical and subtropical regions of the world. Its high water management efficiency during drought are hinged on the plant's capacity to develop thin deep roots that extract subsoil water, close stomata before water stress signs are elicited in the plant and reduce the production of osmolytes which would have otherwise increase water loss from cells during drought (Daryanto *et al*., 2016). The authors also reported increased synthesis of abscisic acid, a compound that lowers leaf area by leaves shedding, limit formation of new leaves and only allows for production of small leaves which concomitant reduction in water loss.

19. The crop is grown for its high starch content (38%) and because it is one of the cheapest source of energy (Howeler*et al*. 2013). Although cassava root contains small amount of calcium (16mg/kg), phosphorus (27mg/kg) and vitamin C (20.6mg/kg), it is low in protein (1%) and other nutrients (Olumide, 2004) such that fortifications with minerals and vitamins and supplementation with proteins is required for balanced animal diet. However, the high amylopectin content (83%) of cassava starch gives it a digestibility of over 75% (Olumide, 2004). Like other crops, cassava has two toxic antinutritional factors. The toxic principles are cyanogenicglucosides; linamarin and lotaustralin which are hydrolysed to hydrocyanic acid that has been associated with acute cyanide intoxication, goiters and chronic pancreatitis.

20. The chemical composition of Cassava (*Manihotesculanta*Crantz) makes it an important food energy supply for human beings and animals. It is the staple food and source of nourishment for more than one billion people worldwide (FAO, 2011) especially in Africa, Asia and South America.Of the 254, 999, 000 tons of cassava produced in 2013 in the world, 39.5% of it (100, 637, 000 tons) was consumed by human beings (FAOSTAT). In Africa, as much as 70% of the cassava produced is for human consumption while between 35 and 40%, and 41% of cassava produced in Latin America and Caribbean, and Asia respectively are used for direct human consumption (Anyanwu*et al*. 2015). It is consumed by human beings in these continents in boiled, fried, roasted, grilled and baked forms as sandwiches, snacks, recipes of soup, desserts and bread (Table 1). The popular fermented products of cassava as shown in same table are alcoholic beverages, *gari, lafun* and*fufu* (*mwanga* or *ugali*)

21. Large quantities of cassava starch are mash with protein concentrates and mineral salts for livestock feeding. FAOSTAT estimates that 34.1% (87,059, 000 tons) of world cassava production of 2013 was used for feed productionand 67130 tons of cassava valued at 39 billion USD was exported to various countries for livestock product trade in 2012.

[9] REP13/CAC para 32

22. The paradigm shift from being only a food crop to an export commodity foruseas an energy crop and global industrial raw material has led to almost 400% increase in world cassava production from 71,259,839 tons in 1961 to 277,102,564 tons in 2016with 15.1% and 14.1% involved in import and export trade respectively (FAOSTAT). Same source of information estimates that as of 2014, the world net production value of cassava was over six billion US dollars. About 15% of world production of 2013 was used for industrial purposes. Cassava starch is used in the manufacture of food, adhesives, thickening agents, paper and pharmaceutical. It is processed to sweeteners mainly glucose, high fructose syrup and sorbitol. Cassava starch and waste (peels) are used in production of ethanol and animal feeds. Cassava waste are increasingly digested to biogas and the waste product of the process used as fertilizer.

23. The increasing need for cassava as both a food crop that is drought resistant and therefore future crop against hunger in adverse climate change conditions, and its current high industrial profile, coupled with the threats to its production and utilization due to hydrocyanic acid and probably mycotoxins has drawn more attention to the crop. Since there is an existing ML for gari and none for other fermented cassava foods, and the limited information on mycotoxins in cassava products; makes the need to establish MLs for fermented cassava foods; (fufu and fufu powder, dried cassava chips and other cassava products: Lafun, Atteke, Chikwangue, Bila, Farinha, Bikedi and NtobaMbodi) and to determine whether mycotoxins are of health concern in consumption of these food commodities an imperative.

Methodology Adopted by EWG

24. This discussion paper therefore intends to generate current data on the incidence and concentrations of HCN and mycotoxins in the fermented cassava products, and the dietary exposure to these contaminants with a view to making appropriate recommendations to CCCF 12 on possibility of establishing MLs for other fermented food products apart from gari and adverse public health effects of mycotoxins in the cassava products.

    If the sourced information on levels of HCN in the fermented cassava products andestimates of their dietary exposure indicates exceedance of ARfD or PMTDI, there will be need to recommend MLs for the contaminant in such product(s). The Provisional Maximum Tolerable Daily Intake (PMTDI) is 0.02 mg/kg body weight while the ML of 2.00mg/kg free hydrocyanic acid is for gari.

25. Similarly, any mycotoxin found at levels above set ML in the fermented products and will therefore lead to exceedance of ARfD and PMTDI will be termed to be of public health concern.

General Information on Hydrocyanic Acid

26. Hydrogen cyanide is a colourless or pale blue liquid or gas with a faint bitter almond-like odour, which is released into the atmosphere from natural biogenic processes either from higher plants, bacteria, and fungi(Orjiekwe*et al*., 2013).

27. Hydrogen cyanide can be producedfollowing the hydrolysis of cyanogenicglucosides; linamarin and a small amount of Lotaustralin present in cassava.The Linamarin is readily hydrolysed to glucose and acetone cyanohydrin in the presence of the enzyme Linamarase, which is also produced by the plant (Orjiekwe*et al*., 2013). The acetone cyanohydrin decomposes rapidly in neutral or alkaline conditions liberating hydrogen cyanide and acetone as shown below

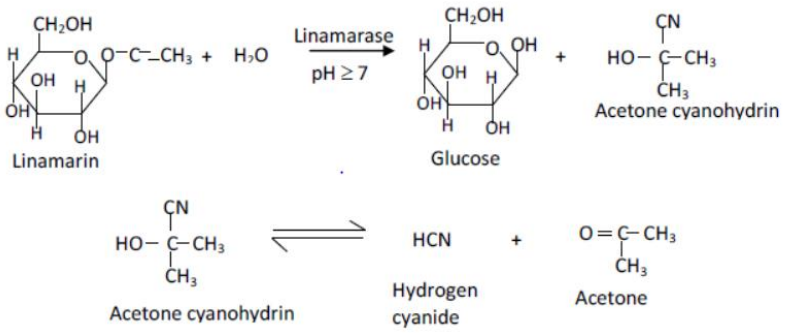

Figure 1: Enzymatic hydrolysis of linamarin. Source: Orjiekwe*et al*., 2013

28. The mechanism involves a two-step reaction which are; the penetration of cyanide into a protein crevice, with initial binding of cyanide to protein while the second step is the binding of cyanide to heme iron. Cyanide exerts its toxic effects by binding to the ferric ion of cytochrome c oxidase, an enzyme that accounts for about 90 percent of the total oxygen uptake in most cells via the electronic

transport chain (Baskin *et al*., 2004). Cyanide acts as an inhibitor of the electron transport chain by binding non-competitively to cytochrome c oxidase (complex IV) and altering the shape of its active site. Consequently electrons cannot be released to oxygen and the electron transport chain shuts down (Garett and Grisham, 2005) thereby resulting in decrease in the utilization of oxygen in the tissues. It also causes an increase in blood glucose and lactic acid levels and a decrease in the ATP/ADP ratio indicating a shift from aerobic to anaerobic metabolism (WHO, 1993).

29. Three main factors determine the degree to which HCN exerts its effect; amount of exposure, route of exposure, and the length of time of the exposure (MDOCH, 2004). HCN poisoning occurs in two forms; acute and chronic poisoning. In acute poisoning short-term exposures to low levels of cyanide through inhalation, skin absorption or ingestion results in rapid breathing and heart rate, restlessness, dizziness, weakness, headache, and nausea/vomiting in few minutes (Mburu, 2013).While in chronic conditions following long term exposure to low levels of cyanide may result in breathing difficulties, eye irritation, chest and/or heart pain, vomiting, loss of appetite, headaches, nosebleeds, enlargement of the thyroid gland (goiter) and death. Tropical AtaxicNeuropathy (TAN) also is a syndrome attributed to dietary cyanide exposure from inadequately prepared cassava. TAN is a progressive disorder that mainly affects older adults (CCDN News, 2008).Survivors of chronic cyanide exposure may develop damage to the brain and the heart and in some cases injury to the central nervous system due to protracted oxygen deprivation to the organ system (Baskin *et al*., 2004).An occurrence of ataxic polyneuropathy was recorded in Ososa, Southwest Nigeria with 22 per 1000 in 1969, 60 per 1000 in 1998 and 64 per 1000 in 2003 (Oluwole*et al*., 2013) which is been attributed to cyanide poisoning from cassava food products. A case was reported in Malaysia due to cassava (ubikayu) poisoning following its consumption amongst a mother and three daughters with the loss of one daughter with symptoms such as nausea, abdominal cramp, diarrhea, vomiting, drowsiness (Arriffin*et al*., 1992). Poor/coloured vision was recorded due to consumption of small amounts of cyanide over a long period of time due to inadequate processing of cassava roots to gari in Zaria metropolis town Nigeria, which contributes to high prevalence of blindness and severe visual impairment (Yusuf *et al*., 2014).

30. The level of hydrocyanic acid (HCN) in cassava limits the use of cassava and its products for livestock feed/food and hence requires additional supplementation of cassava-based diets with methionine and lysine either in its pure form or as animal protein supplements, particularly fish meal(FAOSTAT, 2013).

31. Cyanide levels can be significantly reduced and in some cases eliminated depending on the processing methods employed (FOA, 2004).The processing methods generally adopted include a combination of procedures, such as peeling, slicing, fermentation, boiling, drying, pounding or milling and sieving. Fermentation has shown to reduce and in some cases totally eliminated HCN in cassava products (loop fermentation) (Egwim *et al*., 2013).

    The cassava is first peeled (as about 60-70% of the poison is in the peel) and then soaked in stagnant water or fermented in sacks for about three days. It is sometimes grated or rasped as this helps to speed up the fermentation process (Milena *et al.,* 2013). At the beginning of the fermentation, *Geotricum candidia* acts on the cassava. This helps to make the product acidic, which finally kills off the microorganisms as they cannot exist in such a medium. A second strain of microorganisms (*Corynebacterium lactis*) which can tolerate the acidic environment then take over and by the third day 90-95% of the dangerous chemicals would have been hydrolyzed. The cassava also develops its characteristic flavour. The product is then sieved and the fine starch particles are fried in an iron pan over aflame or with some oil. During this process most, if not all the remaining HCNis released. The liquor from a previous fermentation is used as a starter, thereby reducing the period of fermentation to about 6- 8hours (Egwim *et al*., 2013).

32. Loop fermentation is achieved by using starter culture from already fermented product to inoculate a fresh barge of fermentation process. In this case organisms are “trained” to further utilize the compounds in the fermenting substrate and acidified by squeezing lime (citrus) juice into it before inoculation begins (Egwim *et al*., 2013).

Concentrations of and Dietary Exposure to HCN Hydrocyanic Acid in Fermented Cassava

33. The concentrations of HCN in *gari*, *fufu* (*akpu*) from Nigeria and Sierra Leone (Table 2) available in nine publications were mostly above the ML of 2.00 mg/kg in the products. However, very recent studies (Table 2.1) suggests a high incidence but low levels as of the 65/71 gari samples contaminated, one sample was above 2.0 mg/kg. as expected, 9/10 ready-to-eat cassava flour had HCN, no fufu sample of 10 analysed had HCN. Literature search by the EWG did not reveal data on dietary exposure to HCN.

Evaluation of Existing MLs in Relation to Dietary Exposure to HCN in Fermented Cassava Products

No submissions from members of EWG and other CCCF members on existing MLs of HCN in Fermented

Cassava

Need and Feasibility to Establish ML for HCN in All Fermented Cassava Products

This is deferred to JECFA based on the response received on its data request call

Harmonizing the Expression of HCN Levels-

Suggestions with justification on harmonizing the expression of HCN levelsi.e. free or total HCN are outstanding.

Levels of Mycotoxins in Some Fermented Cassava Products

Gari

The data obtained for gari showed that total aflatoxins were found in 22.64% (113 / 414) of samples analysed, within the range 0.05-13.8 µg/kg (Table 4.1). Aflatoxin B1 was found in 23.5% (19/81) of analysed samples at the mean concentration of 0.25 µg/kg. Ochratoxin A was reported in 18.9% of samples (10/53). Deoxynivalenol(DON) was present in 13.8% (9/65) samples were contaminated within 35-99 µg/kg and at a mean of 57 µg/kg, Fumonisin B1 was implicated in 13% (10/77)of samples within 45-80 µg/kg and mean value of 6 µg/kg. Zearalenone (ZEN) contaminated 79.2% (42/53) of the samples within 11-17µg/kg. Over 40 other mycotoxins reported are found in Appendix 1..

Lafun

There was no report on aflatoxin in lafun however, DON, FB1and ZEN contaminated 36 samples of lafunby 27.8% (31-91 µg/kg), 44% (44-256 µg/kg) and 5.6% (13-16µg/kg), respectively. Thus in comparison with gari it turns out being lower incidence of DON in lafun, higher incidence but lower level of FB1 in lafun, and lower incidence and levels of ZEN in lafun. Other mycotoxins reported in lafun are 15ADON, FB2, DAS, FUS-X and a-zearalenol.

Fufu

Total aflatoxin, fumonisin and ochratoxin were reported in 30 samples of fufu. Their incidence was 90%, 40% and 50% respectively.

Health Implications of Incidence and Levels of Mycotoxins found in the Fermented Cassava Products

The incidence of aflatoxins in gari and lafun consumed across Nigeria is indicative of the possibility of chronic or acute toxicological impacts. Liver cancer, liver cirrhosis, immunosuppression, growth impairment, mutagenesis, and death have been attributed to ingestion of aflatoxin contaminated food or food product. Aflatoxin contamination of over 200µg/kg in maize led to the death of over 106 Western Indians and 125 Kenya natives (Bhumi and Chinnam, 2007; Azziz-Baumgartner *et al*., 2005). Also, Immune suppression in the Gambia (Turner *et al*., 2003) along with have child stunting and underweight in children inTogo, Benin and Tanzania (Gong *et al*, 2004) have also been reported.

The mycotoxin profiles in the various works reported in this discussion paper showed that the mycotoxin contaminants did not occur singly but in combinations of twos, threes, fours and fives and more. The implications of such toxin "cocktails" on human health could be synergistic, additive or antagonistic in host organisms (Miller, 1995). Interaction between AFB1 and FB1, which is one of the combinations observed, had an additive effect in mice, causing increased injuries to liver and kidneys of the experimental animals (Gelderblom et al., 2002). Other combinations which were observed in the work and have been demonstrated by other workers to exhibit synergistic interactions include AFB1 and the trichothecenes (Placinta et al., 1999), FB1 and OTA (Creppy et al., 2004), and FB1 and ZEA (Luongo et al., 2008). The simultaneous exposure of OTA and AFB1 to rabbits demonstrated an antagonistic interaction between the toxins with regards to teratogenic effects (Wangikar et al., 2005). The complex and varied nature of the effects of mixed mycotoxins is obvious in the synergistic and additive growth depression effects of DON and FB1 in pigs and broiler chicks respectively (Placinta et al., 1999). DON is antagonistic to T-2 in the inhibition of human lymphocytes proliferation (Speijer and Speijer 2004). The interaction data between four or more mycotoxin species, a recurring feature in the rice samples, are virtually unavailable; however, Speijer and Speijer (2004) postulated that combined exposure to several classes of mycotoxins generally results in an additive effect with a few minor exceptions, indicating synergistic interaction.

While dietary exposure to mycotoxins is yet to be examined, however from the values of the toxins found mostly in Nigeria, it is unlikely that acute effects could occur from a sole source of cassava, although a long term health impacts may be fueled.

FAO food security data of 2012 reveals that an average Nigerian, based on 2005 - 2007 survey consumes cassava at 321.89 g/person/day (FAOSTAT, 2012 - http://nso.nigeria.opendataforafrica.org/bpggoxe/fao-food-security-data-by-food-groups-items-june-2012) making up 10% of food item consumed.

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Annex I

Table 1: Cassava Based Food Products from Various Countries of the World

Continent	Country	Name of non-fermented food products	Name of Fermented food product
South America	Bolivia	Boiled and fried Yuca	
	Brazil	Cooked root to paste-*Vaca-atolada* *Pirão*- gravy-like gruel prepared by cooking fish bits (such as heads and bones) with cassava flour. *Farofa*-lightly roasted cassava Cassava cake Boiled cassava into sweet pudding	
	Colombia	*Sancocho*-Soup *Pandebono* bread from *Yuca* dough *Bollo de yuca*-boiled dough served with butter and cheese. *Enyucados*-dessert from ground boiled *yuca* Boiled, fried and roasted cassava	
	Suriname	*Telo*-steamed and deep fried cassava withed salted fish.	
	Ecuador	Boiled, *Yuquitos*-fried chips, *Bolitos de yuca*-bread, yuca dough, baked yuca dough.	*Chicha*-traditional fermented drink
	Paraguay	Boiled and *Chipa*-bagel-shaped cheesy bread	
	Peru	Boiled and fried *yucca*	
	Venezuela	Boiled, fried and grilled yuca *Plancha*-roasted ground cassava spread as pancake. *Casabe*-pancake	
Central America	Belize	*Bammy*-fried cassava cake. Cassava pane-dessert recipe Component of *Bile up.* *Ereba*-Cassava bread	
	El Salvador	Yuca used in soup and sandwiches *YucaFrita con Chicharrón*-deep fried yuca	
	Costa Rica	Boiled, fried and as snacks	
	Panama	*Carimanolas*-boiled, mashed cassava dough	
	Nicaragua	As soup and recipe of *vigoron* and *vaho* dishes	
Caribbean	Cuba	*Casabe*-round shaped flat bread *Yucafrita*-similar to French fries. Ingredient of Cuban vegetarian stew (*Ajiaco*) and Cuban *Buñuelos,* a local variation of a traditional Spanish fritter	
	Haiti	Eaten as bread, flour boiled into a meal named *Moussa*, various soups referred to as *joumou* and cookies called *BonBonLamindon*	
	Dominican Republic	Used as cassava bread (*casaba*), french fries (*arepitas de yuca),* dough made from cassava flour (*catibía*) and fried grated ((*chulos).*	
	Puerto Rico	Used to cook stew (*Sancocho),* eaten as boiled, in form of paste (*masa),* and other dishes (*pasteles* and *alcapurrias*)	
	Jamaica	Cassava cake (*Bammy*)	
	Bahamas	Eaten boiled, cooked in soup with okra and baked into cakes	
	Eastern	Boiled and served with flour dumplings and	

Continent	Country	Name of non-fermented food products	Name of Fermented food product
	Caribbean	other root vegetables	
	Bermuda	Cassava pie and cassava chips (for export)	
Africa	Nigeria and Sierra Leone	Boiled cassava	*Eba or gari, lafun and fufu*
	Central Africa	Eaten as boiled, mashed and cooked and snack.	
	Tanzania and Kenya	Fried, roasted and cassava flour made into *ugali* or *mwanga* or *fufu.*	*ugali* or *mwanga* or *fufu.*
	Central African Republic	Fries, boiled, snacks and bread	
Asia	China	Production of ethanol/export trade	
	India	Boiled, deep fried to make crisps and used to sweet milk puddings.	
	Indonesia	Eaten as boiled, fried or baked.	It is fermented to make *peuyeum* and *tape,* a sweet paste which can be mixed with sugar and made into a drink, the alcoholic (and green) *es tape.*
	Philippines	Steamed and eaten plain, as dessert	
	Sri Lanka	Eaten as supplementary diet in boiled form	
	Vietnam	Tapioca	

Annex II

Table 2: Occurrence and Concentrations of Hydrocyanic Acid in Fermented Cassava Products

Fermented Cassava Product	Country / Location	Number of contaminated sample/total number of samples analyzed	Range of concentration (mg/kg)	Mean ± SD	No of samples above ML for HCN of 2mg/kg	Limit of detection of method	Reference
Garri	Nigeria Okada town, Edo State,	12/12	5-10mg/kg	5±0.10	12		Orjiekwe*et al*, 2013
Fufu	Nigeria Okada town, Edo State	12/12	5-10mg/kg	10±0.13	12		Orjiekwe*et al*, 2013
White garri (processed by researcher) White gari (purchased from market)	Nigeria Osusu, IsialaNgwa, Abia State	1/1 20/20	3.8-32.2mg/kg 3.70-65.8g/kg	3.8±0.6 24.21±17.55	1 20		Odoemelam, 2005
Yellow gari Yellow gari (purchased from market)	Nigeria Osusu, IsialaNgwa,Abia State	6/6 20/20	0.62-20.3mg/kg 1.8-52.1mg/kg	0.62±0.6 14.35±13.85	5 19		Odoemelam, 2005
Gari	Nigeria Ekiti State	6/6	2.10-15.30mg/kg	8.41±4.75	6		Babalola, 2014
Gari (urban areas) Rural areas	Nigeria Ekiti,Oyo, Lagos, Ondo and Osun states	10/10 10/10	0.03-0.11mg/kg 0.01-0.08mg/kg	0.07±0.03 0.03±0.02	0 0		Abimbola, 2012
Akpu	Nigeria Karu, Nasarawa state	2/2	2.04-8.54mg/kg	2.04±0.64	2		Ojo*et al*, 2013
Gari	Nigeria Karu, Nasarawa state	2/2	2.04-8.54mg/kg	8.54±0.30	2		Ojo*et al*, 2013
Gari	Nigeria Oshodi, Lagos	4/154	3-200 mg/kg	39.15±38.75	2		NAFDAC, 2017
Soaked cassava	Fiji Islands Tonga,Vanuatu and Fiji	10/10	<0.1	<0.1	0		Dolodolotawake*et al*, 2011
Fermented cassava mash Fufu	Nigeria Uyo, AkwaIbom state		8.43-10.73mg/kg	8.43±2.03 10.73±2.03	All all		Uyoh*et al*, 2009
Foofoo Gari	Sierra Leone Freetown,	51/51 36/36		28.2±21.2 8.6±3.45	51 36		Blanshard*et al*, 1993

***Table 2.1: Update on** Occurrence and Concentrations of Hydrocyanic Acid in Fermented Cassava Products*

Fermented Cassava Product	***Country / Location***	***Number of contaminated sample/total number of samples analyzed***	***Range of concentration (**mg/kg)***	***Mean ± SD***	***No of samples above ML for HCN of 2mg/kg***	***Limit of detection of method (mg/kg)***	***Reference***
Gari	***Nigeria*** *Edo State, Anambra State, Ondo state*	*11/11*	***0.221 – 1.935***	*1.289±0.603*	***0***	***0.01***	*Olorunnadoet al., 2019 Unpublished*
Gari	***Nigeria*** *Kogi state, and Ekiti state*	***24/24***	***0.056 – 1.593***	*1.048±0.388*	***0***	***0.01***	*Olorunnadoet al., 2019 Unpublished*
Gari	***Nigeria*** *Niger state*	***11/12***	*0.168 - 0.174*	*1.198±0.428*	***0***	***0.01***	*Olorunnadoet al., 2019 Unpublished*
Gari	***Nigeria*** *Bauchi (west)*	***5/5***	***1.315 – 2.463***	*1.613±0.480*	***1***	***0.01***	*Olorunnadoet al., 2019 Unpublished*
Gari	***Nigeria*** *Kaduna state*	***9/9***	***0.734 -*** *1.508*	*1.129±0.240*	***0***	***0.01***	*Olorunnadoet al., 2019 Unpublished*
Gari	***Nigeria Kogi state***	***5/10***	***0.441-1.838***	*1.106±0.549*	***0***	*0.03*	*Apehet al., 2019 Unpublished*
Fufu	***Nigeria Kogi state***	***0/10***	***-***	-	***0***	*0.03*	*Apeh et al., 2019 Unpublished*
Cassava flour	***Nigeria Kogi state***	***9/10***	***0.309-6.294***	*2.639±1.836*	***6***	*0.03*	*Apeh et al., 2019 Unpublished*

Table *2.2: Occurrence of Cyanogenic glycosides (linamarin and lotaustralin) in fresh and dried cassava*

	Sample Type	**Country / Location**	**Number of contaminated sample/total number of samples analyzed**	**Range of concentration (**µg/kg)	**Mean (**µg/kg)	**No of samples above ML**	**Limit of detection of method (**µg/kg)	**Reference**
Linamarin	Dry Cassava	Mali Bamako	2/2	512.249-557.687	534.968		2.30	Ingenbleek *et al*., 2019
		Mali Sikasso	2/2	741.582-991.062	866.322		2.30	Ingenbleek *et al*., 2019
		Benin Tchaourou	2/2	295.776-335.984	315.880		2.30	Ingenbleek *et al*., 2019
		Benin Cotonou	2/2	1654.628-5863.215	3758.921		2.30	Ingenbleek *et al*., 2019
		Cameroon Duala	2/2	1024.498-18440.963	9732.730		2.30	Ingenbleek *et al*., 2019
		Nigeria Lagos	2/2	154.145-3532.160	1843.153		2.30	Ingenbleek *et al*., 2019
	Fresh Cassava	Cameroon Duala	2/2	92590.608-198126.755	145358.681		2.30	Ingenbleek *et al*., 2019
		Mali Sikasso	2/2	97820.263-316951.368	207385.816		2.30	Ingenbleek *et al*., 2019
		Benin Tchaourou	2/2	100992.358-262597.252	181974.800		2.30	Ingenbleek *et al*., 2019
		Benin Cotonou	0/1	0			2.30	Ingenbleek *et al*., 2019
		Nigeria Lagos	0/1	0			2.30	Ingenbleek *et al*., 2019
Lotaustralin	Dry Cassava	Mali Bamako	1/2	153.624			1.30	Ingenbleek *et al*., 2019
		Mali Sikasso	1/2	585.861			1.30	Ingenbleek *et al*., 2019
		Benin Cotonou	1/2	303.764			1.30	Ingenbleek *et al*., 2019

		Benin Tchaourou	1/2	40.913			1.30	Ingenbleek *et al*., 2019
		Cameroon Duala	1/2	118.872			1.30	Ingenbleek *et al*., 2019
		Nigeria Lagos	1/2	662.843			1.30	Ingenbleek *et al*., 2019
	Fresh Cassava	Cameroon Duala	1/2	13017.249			1.30	Ingenbleek *et al*., 2019
		Mali Sikasso	1/2	18395.786			1.30	Ingenbleek *et al*., 2019
		Benin Tchaourou	1/2	17384.655			1.30	Ingenbleek *et al*., 2019

Annex III

Table 3: Occurrence and concentrations of mycotoxins in fermented cassava products

Fermented Cassava Product	Type of mycotoxin	Country / Location	Number of contaminated sample/total number of samples analyzed	Range of concentration	Mean ± SD	No of samples above ML for Myco toxin	Limit of detection of method	Reference
Gari	DON	Nigeria	9/24	35-99	57±19		14.5	Chilaka *et al*., 2017
	DON-3G	Nigeria	3/24	12-20	16±5		3.2	Chilaka *et al*., 2017
	FB1	Nigeria	6/24	45-80	6±13		15.0	Chilaka *et al*., 2017
	FB2	Nigeria	5/24	29-65	40±15		10.5	Chilaka *et al*., 2017
	ZEN	Nigeria	4/24	11-17	14±7		3.6	Chilaka *et al*., 2021
	DAS	Nigeria	2/24	5-10	8±3		2.0	Chilaka *et al*., 2017
	T-2	Nigeria	3/24	17-22	19±3		4.5	Chilaka *et al*., 2017
	Total Aflatoxin	Nigeria	11/24	0.05-3.3	0.74± 1.03		1	NAFDAC, 2015
	Total Aflatoxin	Nigeria	18/46	1.1-13.8	1.10± 2.26		1	NAFDAC, 2016
	Total Aflatoxin	Nigeria	24/34	1.0-5.4	1.73± 1.43		1	NAFDAC, 2017
	AFB1	Nigeria Benin City,	3/10	1500-2000				Ibeh *et al*., 1991
	Ochratoxin	Nigeria Niger state,	18/18	3.28-22.73	7.63± 4.07	10	0.001	Makun *et al*., 2013
	Total Aflatoxin	Nigeria Anambra	6/30	0.44–3.69				Ogiehor *et al*., 2007
	Total Aflatoxin	Nigeria Cross River	5/30	0.32–4.57				Ogiehor *et al*., 2007
	Total Aflatoxin	Nigeria Delta	3/30	0.26–3.64				Ogiehor *et al*., 2007
	Total Aflatoxin	Nigeria Edo	4/30	0.13–4.46				Ogiehor *et al*., 2007
	Total Aflatoxin	Nigeria Enugu	5/30	0.37–5.71				Ogiehor *et al*., 2007
	Total Aflatoxin	Nigeria Imo	7/30	0.14–3.16				Ogiehor *et al*., 2007
	Total Aflatoxin	Nigeria Lagos	9/30	0.12–2.54				Ogiehor *et al*., 2007

Fermented Cassava Product	Type of mycotoxin	Country / Location	Number of contaminated sample/total number of samples analyzed	Range of concentration	Mean ± SD	No of samples above ML for Myco toxin	Limit of detection of method	Reference
	Total Aflatoxin	Nigeria Ogun	3/30	0.25–1.66				Ogiehor *et al*., 2007
	Total Aflatoxin	Nigeria Ondo	2/30	0.18–2.41				Ogiehor *et al*., 2007
	Total Aflatoxin	Nigeria Rivers	8/30	0.17–4.14				Ogiehor *et al*., 2007
	AFB1	Nigeria	13/18	nd-0.69	0.25	0	0.5 ng/ml	Adejumo *et al*., 2013
Lafun	DON	Nigeria	10/36	31-91	62±19		14.5	Chilaka *et al*., 2017
	15ADON	Nigeria	3/36	21-36	30±8		8.5	Chilaka *et al*., 2017
	FB1	Nigeria	16/36	44-256	110± 71		15.0	Chilaka *et al*., 2017
	FB2	Nigeria	22/36	30-392	116± 89		10.5	Chilaka *et al*., 2017
	ZEN	Nigeria	2/36	13-16	15±2		3.6	Chilaka *et al*., 2017
	DAS	Nigeria	11/36	7-22	14±6		2.0	Chilaka *et al*., 2017
	FUS-X	Nigeria	3/36	128-159	143± 16		54.5	Chilaka *et al*., 2017
	a-zearalenol	Nigeria Lagos,	1/1	11			6	Rubert *et al*., 2013
Cassava chips/ flour	Total Aflatoxin	Kenya Nairobi and Mombasa,	3/36	2.84-8.89	6.11± 3.05	2	1	Gacheru Patrick *et al*., 2015
	Total Aflatoxin	Nigeria Ogun,	3/4	0.07-0.07	0.05			Adejumo *et al*., 2013
Cassava chips	Total Aflatoxin	Cameroun	18/72	5.2- 14.5				Essono *et al*., 2009
		Uganda / Ngora	4/15	0-3.5	0.633±			Kaaya and Eboku, 2010
		Uganda / Kumil	6/17	0-2.5	0.412±			
		Uganda / Bukedea	8/28	0-4.5	0.500±			
		Rwanda	0/15	nd				Matsiko *et al*., 2017

Table 3.1: Update on Occurrence of Mycotoxin in Fermented Cassava Products

Fermented Cassava Product	*Type of mycotoxin*	*Country (location)*	*Number of contaminated sample / Total number of samples analyzed*	*Range of concentration*	*Mean ± SD*	*No. of samples above ML for Mycotoxin*	*ML (ppb) EU*	*Limit of detection of method*	*Reference*
Gari	*Aflatoxin B1 (AFB1)*	*Nigeria*	*2 /41*	*0.043 – 0.265*	*0.154 ±0.157*	*0*	*2*	*0.003*	*Olorunnadoet al., 2019 Unpublished*
	AFB2	*Nigeria*	*34 / 41*	*0.028-1.994*	*0.409 ±0.386*	*0*		*0.021*	*Olorunnadoet al., 2019 Unpublished*
	AFG1	*Nigeria*	*6/41*	*0.035 – 0.076*	*0.0545±0.014*	*0*		*0.013*	*Olorunnadoet al., 2019 Unpublished*
	AFG2	*Nigeria*	*1 /41*	*0.07*	*0.07*	*0*		*0.06*	*Olorunnadoet al., 2019 Unpublished*
	Ochratoxin A (OTA)	*Nigeria*	*9 /41*	*0.011-0.089*	*0.028 ±0.024*	*0*	*3*	*0.001*	*Olorunnadoet al., 2019 Unpublished*
	OTB	*Nigeria*	*21/ 41*	*0.002-0.021*	*0.0044 ±0.005*	*0*		*0.001*	*Olorunnadoet al., 2019 Unpublished*
	Fumonisin B1	*Nigeria*	*1 /41*		*6.117*	*0*	*1000*	*1.738*	*Olorunnadoet al., 2019 Unpublished*
	FumonisinB2	*Nigeria*	*1 /41*		*8.1*	*0*	*1000*	*0.033*	*Olorunnadoet al., 2019 Unpublished*
	FumonisinB3	*Nigeria*	*1 /41*		*3.973*	*0*		*0.47*	*Olorunnadoet al., 2019 Unpublished*
	Zearalenol(ZEA)	*Nigeria*	*40/ 41*	*0.082-5.869*	*1.0295 ±1.218*	*0*	*75*	*0.11*	*Olorunnadoet al., 2019 Unpublished*
	α-ZEA	*Nigeria*	*40/ 41*	*0.916-19.634*	*4.1819 ±3.281*	*0*		*0.243*	*Olorunnadoet al., 2019 Unpublished*
	β-ZEA	*Nigeria*	*41/ 41*	*0.315-2.454*	*0.9344 ±0.562*	*0*		*0.697*	*Olorunnadoet al., 2019 Unpublished*
	HT-2 Toxin	*Nigeria*	*7 /41*	*0.351 – 2.768*	*0.9031 ±0.834*	*0*	*50*	*0*	*Olorunnadoet al., 2019 Unpublished*
	Deoxynivalenol	*Nigeria*	*0 /41*	*-*	*-*	*0*	*750*	*3.633*	*Olorunnadoet al., 2019 Unpublished*
	15-Acetyl-Deoxynivalenol	*Nigeria*	*1 /41*		*5.098*	*0*		*2.964*	*Olorunnadoet al., 2019 Unpublished*
	3-Acetyldeoxynivalenol	*Nigeria*	*9 /41*	*0.182 – 0.408*	*0.2755 ±0.075*	*0*		*0.078*	*Olorunnadoet al., 2019 Unpublished*
	Kojic Acid	*Nigeria*	*39/ 41*	*3.053-381.398*	*110.395 ±76.249*	*0*		*2.934*	*Olorunnadoet al., 2019 Unpublished*
	Nivalenol	*Nigeria*	*0 /41*	*-*	*-*			*0.01*	*Olorunnadoet al., 2019 Unpublished*
	Tenuazonic copper acid	*Nigeria*	*4 /41*	*0.069 - 0.6*	*0.1917 ±0.107*	*0*		*0.059*	*Olorunnadoet al.,*

									2019 Unpublished
	Citrinin	Nigeria	40/ 41	0.121-1.422	0.5864±0.36	0	2000	0.011	Olorunnadoet al., 2019 Unpublished
	Sterigmatocystine	Nigeria	7 /41	0.057-0.3240	0.1251±0.091	0		0.047	Olorunnadoet al., 2019 Unpublished
	Cyclopiazonic Acid	Nigeria	6 /41	0.008-0.069	0.0253 ±0.024	0		0.007	Olorunnadoet al., 2019 Unpublished
	Alternariolmono methyl ether (AME)	Nigeria	41/ 41	0.088-0.974	0.3139 ±0.210			0.888	Olorunnadoet al., 2019 Unpublished
Gari	Total Aflatoxin	Kogi, North Central Nigeria	8 /10	0.921-5.307	3.371±0.249	3	4	0.002	Apeh et al., 2019 Unpublished
	Fumonisin	Kogi, North Central Nigeria	3 /10	0.006-0.146	0.249±0.308	0	1000	0.002	Apeh et al., 2019 Unpublished
	Ochratoxin	Kogi, North Central Nigeria	1 /10	0-12.435	12.435	1	3	0.002	Apeh et al., 2019 Unpublished
Fufu	Total Aflatoxin	Kogi, North Central Nigeria	9 /10	0.913-3.634	2.201±0.783	0		0.002	Apeh et al., 2019 Unpublished
	Fumonisin	Kogi, North Central Nigeria	4 /10	0.831-2.054	1.289±0.5410	0	1000	0.002	Apeh et al., 2019 Unpublished
	Ochratoxin	Kogi, North Central Nigeria	5 /10	0.354-9.480	3.927±3.808	3	3	0.002	Apeh et al., 2019 Unpublished
Cassava fluor (non-fermented)	Total Aflatoxin	Kogi, North Central Nigeria	10/ 10	0.363-3.680	2.596±.1.070	0		0.002	Apeh et al., 2019 Unpublished
	Fumonisin	Kogi, North Central Nigeria	0 /10	0.00	-	0		0.002	Apeh et al., 2019 Unpublished
	Ochratoxin	Kogi, North Central Nigeria	2 /10	1.813-4.337	3.075±1.785	0	5	0.002	Apeh et al., 2019 Unpublished
Dried Cassava product (gari, lafun etc)	Aflatoxin B1	Cotonou Benin	1/ 12	1.72	1.72	0		0.1	Ingenbleek et al., 2019
	Ochratoxin A	Cotonou Benin	1/ 12	0.79	0.79	0		0.1	Ingenbleek et al., 2019
	Fumonisin b1	Tchaourou Benin, Duala Cameroon	3/ 12	19.90-91.61	44.01 ± 41.23	0		2.5	Ingenbleek et al., 2019
	Fumonisin B2	Tchaourou Benin, Duala Cameroon	1/ 12	24.70	24.70	0		2.2	Ingenbleek et al., 2019
	Fumonisin B3	Duala Cameroon	1/ 12	10.87	10.87			2.2	Ingenbleek et al., 2019
	Fumonisin B4	Duala Cameroon	1/ 12	7.42	7.42			2.2	Ingenbleek et al., 2019
	Zearalenone	Tchaourou Benin, Duala Cameroon	2/ 12	0.72-87.52	44.12 ± 61.38	1		0.2	Ingenbleek et al., 2019
	Citrinin	Tchaourou Benin, Duala Cameroon	2/ 12	38.95-97.33	68.14 ± 41.28	0		0.75	Ingenbleek et al., 2019
	Sterigmatocystin	Bamako Mali	1/ 12	0.34	0.34			0.075	Ingenbleek et al., 2019

	Averufin	Bamako Mali, Tchaourou Benin	3/ 12	0.12-0.50	0.34 ± 0.20			0.021	Ingenbleek et al., 2019
	Kojic acid	Bamako Mali, Sikasso Mali, Lagos Nigeria	5/ 12	99.10-2764.10	1163.94 ± 1090.81			20	Ingenbleek et al., 2019
	3-Nitropropionic acid	Bamako Mali, Duala Cameroon	2/ 12	23.71-119.50	71.61 ± 67.74			0.75	Ingenbleek et al., 2019
	Malformin C	Bamako Mali	1/ 12	0.87	0.87			0.11	Ingenbleek et al., 2019
	Nivalenol	Duala Cameroon	1/ 12	52.59	52.59			0.8	Ingenbleek et al., 2019
	Moniliformin	Tchaourou Benin, Duala Cameroon, Sikasso Mali, Bamako Mali	5/ 12	6.29-441.42	22.76 ± 16.78			1.5	Ingenbleek et al., 2019
	Culmorin	Duala Cameroon	1/ 12	9.67	9.67			1.7	Ingenbleek et al., 2019
	Equisetin	Tchaourou Benin, Duala Cameroon	2/ 12	2.39-4.87	3.63 ± 1.76			0.7	Ingenbleek et al., 2019
	Tenuazonic acid	Tchaourou Benin	1/ 12	47.01	47.01			1.0	NIngenbleek et al., 2019
	Macrosporin	Tchaourou Benin, Duala Cameroon	2/ 12	0.51-0.61	0.56 ± 0.07			0.13	Ingenbleek et al., 2019
	Festuclavin	Bamako Mali	1/ 12	0.42	0.42			0.02	Ingenbleek et al., 2019
	Quinolactacin A	Tchaourou Benin, Duala Cameroon, Sikasso Mali	6/ 12	0.07-1.23	0.44 ± 0.48			0.013	Ingenbleek et al., 2019
	Dihydroxymellein	Tchaourou Benin	1/12	9.22	9.22				Ingenbleek et al., 2019
	Flavoglaucin	Cotonou Benin, Tchaourou Benin, Duala Cameroon, Sikasso Mali, Bamako Mali	7 /12	0.30-37.04	8.29 ± 13.40			0.03	Ingenbleek et al., 2019
	Curvularin	Duala Cameroon	1 /12	2.77	2.77			0.6	Ingenbleek et al., 2019
	Berkedrimane B	Sikasso Mali, Bamako Mali	2 /12	4.23-6.54	5.44 ± 1.71			0.4	Ingenbleek et al., 2019
	Purpuride	Sikasso Mali	1 /12	1.93	1.93			0.08	Ingenbleek et al., 2019
Fresh Cassava (Unfermented cassava products)	Ochratoxin A	Duala Cameroon	1/ 8	0.65	0.65	0		0.1	Ingenbleek et al., 2019
	Methylsulochrin	Duala Cameroon	1/ 8	0.30	0.30			0.04	Ingenbleek et al., 2019
	Integracin A	Lagos Nigeria	1/ 8	0.49	0.49			0.04	Ingenbleek et al., 2019
	Integracin B	Lagos Nigeria	1/ 8	0.91	0.91			0.09	Ingenbleek et al., 2019
	Methylfunicone	Lagos Nigeria	1/ 8	0.51	0.51			0.04	Ingenbleek et al., 2019

	Alternariolmethy lether	*Duala Cameroon*	*1/ 8*	*2.41*	*2.41*			*0.15*	*Ingenbleek et al., 2019*
	Quinolactacin A	*Duala Cameroon*	*2/ 8*	*0.07-0.21*	*0.14 ± 0.10*			*0.013*	*Ingenbleek et al., 2019*
	Flavoglaucin	*Tchaourou Benin*	*1/ 8*	*0.51*	*0.51*			*0.03*	*Ingenbleek et al., 2019*
Cooked Cassava	*AFB1*	*USA (Imported)*	*0/40*			*0*		*0.5*	*GEMS Database (Source: USA)*
	AFB2	*USA (Imported)*	*0/40*			*0*		*0.3*	*GEMS Database (Source: USA*
	AFG1	*USA (Imported)*	*0/40*			*0*		*0.7*	*GEMS Database (Source: USA*
	AFG2	*USA (Imported)*	*0/40*			*0*		*0.9*	*GEMS Database (Source: USA*
	DON	*USA (Imported)*	*0/40*			*0*		*10.0*	*GEMS Database (Source: USA*
	FB1	*USA (Imported)*	*0/40*			*0*		*4.0*	*GEMS Database (Source: USA*
	FB2	*USA (Imported)*	*0/40*			*0*		*10.0*	*GEMS Database (Source: USA*
	FB3	*USA (Imported)*	*0/40*			*0*		*8.0*	*GEMS Database (Source: USA*
	OTA	*USA (Imported)*	*0/40*			*0*		*0.8*	*GEMS Database (Source: USA*
	T2	*USA (Imported)*	*0/40*			*0*		*9.0*	*GEMS Database (Source: USA*
	Zea	*USA (Imported)*	*0/40*			*0*		*7.0*	*GEMS Database (Source: USA*
Raw Cassava	*AFB1*	*USA (Imported)*	*0/20*			*0*		*0.5*	*GEMS Database (Source: USA*
	AFB2	*USA (Imported)*	*0/20*			*0*		*0.3*	*GEMS Database (Source: USA*
	AFG1	*USA (Imported)*	*0/20*			*0*		*0.7*	*GEMS Database (Source: USA*
	AFG2	*USA (Imported)*	*0/20*			*0*		*0.9*	*GEMS Database (Source: USA*
	DON	*USA (Imported)*	*0/20*			*0*		*10.0*	*GEMS Database (Source: USA*
	FB1	*USA (Imported)*	*0/20*			*0*		*4.0*	*GEMS Database (Source: USA*
	FB2	*USA (Imported)*	*0/20*			*0*		*10.0*	*GEMS Database (Source: USA*
	FB3	*USA (Imported)*	*0/20*			*0*		*8.0*	*GEMS Database (Source: USA*
	OTA	*USA (Imported)*	*0/20*			*0*		*0.8*	*GEMS Database (Source: USA*
	T2	*USA (Imported)*	*0/20*			*0*		*9.0*	*GEMS Database (Source: USA*
	Zea	*USA (Imported)*	*0/20*			*0*		*7.0*	*GEMS Database (Source: USA*

Table 4.0 Summary of the Cassava Products Analysed for HCN and Mycotoxin Contents within 8 countries

Metabolite	Gari					Fufu					Lafun					Chips					Flour					Fresh / Unfermented					Cooked					Raw				
	TA	TC	TU	PC	PA	TA	TC	TU	PC	PA	TA	TC	TU	PC	PA	TA	TC	TU	PC	PA	TA	TC	TU	PC	PA	TA	TC	TU	PC	PA	TA	TC	TU	PC	PA	TA	TC	TU	PC	PA
Aflatoxin Total	414	113	4	27.3	1.0	10	9	0	90.0	0.0						164	39	19	23.8	48.7	33	13	3	39.4	9.1																
AFB1	81	19	0	23.5	0.0																									40	0	0	0.0	0.0	20	0	0	0.0	0.0
AFB2	41	34	0	82.9	0.0																									40	0	0	0.0	0.0	20	0	0	0.0	0.0
AFG1	41	6	0	14.6	0.0																									40	0	0	0.0	0.0	20	0	0	0.0	0.0
AFG2	41	1	0	2.4	0.0																									40	0	0	0.0	0.0	20	0	0	0.0	0.0
Fumonisin	10	3	0	30.0	0.0	10	4	0	40.0	0.0											10	0	0	0.0	0.0															
Fumonisin B1	77	10	0	13.0	0.0						36	16	0	44.4	0.0															40	0	0	0.0	0.0	20	0	0	0.0	0.0
Fumonisin B2	77	7	0	9.1	0.0						36	22	0	61.1	0.0															40	0	0	0.0	0.0	20	0	0	0.0	0.0
FumonisinB3	53	2	0	3.8	0.0																									40	0	0	0.0	0.0	20	0	0	0.0	0.0
FumonisinB4	12	1	0	8.3	0.0																																		
Ochratoxin	28	19	11	67.9	39.3	10	5	3	50.0	30.0											10	2	0	20.0	0.0															
Ochratoxin A	53	10	0	18.9	0.0																					8	1	0	12.5	0.0	40	0	0	0.0	0.0	20	0	0	0.0	0.0
Ochratoxin B	41	21	0	51.2	0.0																																		
Sterigmatocystine	53	8	0	15.1	0.0																																		
T-2	24	3	0	12.5	0.0																									40	0	0	0.0	0.0	20	0	0	0.0	0.0
HT-2 Toxin	41	7	0	17.1	0.0																																		
ZEN	53	42	1	79.2	1.9						36	2	0	5.6	0.0															40	0	0	0.0	0.0	20	0	0	0.0	0.0
α-ZEA	41	40	0	97.6	0.0						1	1	0	100.0	0.0																								
β-ZEA	41	41	0	100.0	0.0																																		
DON	65	9	0	13.8	0.0						36	10	0	27.8	0.0															40	0	0	0.0	0.0	20	0	0	0.0	0.0
DON-3G	24	3	0	12.5	0.0																																		
15-AC DON	41	1	0	2.4	0.0						36	3		8.3	0.0																								
3- AC DON	41	9	0	22.0	0.0																																		
Nivalenol	53	1	0	1.9	0.0																																		
Citrinin	53	42	0	79.2	0.0																																		
DAS	24	2	0	8.3	0.0						36	11	0	30.6	0.0																								
Kojic Acid	53	44	0	83.0	0.0																																		
Cyclopiazonic Acid	41	6	0	14.6	0.0																																		
Tenuazonic copper acid	53	5	0	9.4	0.0																																		
FUS-X											36	3	0	8.3	0.0																								
Alternariolmethylether	41	41	0	100.0	0.0																					8	1	0	12.5	0.0										
Averufin	12	3	0	25.0	0.0																																		
3-Nitropropionic acid	12	2	0	16.7	0.0																																		
Malformin C	12	1	0	8.3	0.0																																		
Moniliformin	12	5	0	41.7	0.0																																		
Culmorin	12	1	0	8.3	0.0																																		
Equisetin	12	2	0	16.7	0.0																																		
Macrosporin	12	2	0	16.7	0.0																																		
Festuclavin	12	1	0	8.3	0.0																																		
Quinolactacin A	12	6	0	50.0	0.0																					8	2	0	25.0	0.0										
Dihydroxymellein	12	1	0	8.3	0.0																																		
Flavoglaucin	12	7	0	58.3	0.0																					8	1	0	12.5	0.0										
Curvularin	12	1	0	8.3	0.0																																		
Berkedrimane B	12	2	0	16.7	0.0																																		
Purpuride	12	1	0	8.3	0.0																																		
Methylsulochrin																										8	1	0	12.5	0.0										
Integracin A																										8	1	0	12.5	0.0										
Integracin B																										8	1	0	12.5	0.0										
Methylfunicone																										8	1	0	12.5	0.0										
HCN	348	192	104	55.2	29.9	75	65	65	86.7	86.7											10	9	6	90.0	60.0	10	10	0	100.0	0.0										

KEY	
Total Number analysed	TA
Total Number Contaminated	TC
Total Number above ML	TU
% Contaminated	PC
% Above ML	PA

Table 4.1 Total aflatoxin, **HCN and Mycotoxin Contents of Fermented and Non-fermented Cassava products**

S/N	Metabolite	Fermented Cassava Products					Unfermented Cassava Products				
		TA	TC	TU	PC (%)	PA (%)	TA	TC	TU	PC (%)	PA (%)
1.	Aflatoxin Total	424	122	4	28.8	0.9	197	52	22	26.4	11.2
2.	AFB1	81	19	0	23.5	0.0	60	0	0	0.0	0.0
3.	AFB2	41	34	0	82.9	0.0	60	0	0	0.0	0.0
4.	AFG1	41	6	0	14.6	0.0	60	0	0	0.0	0.0
5.	AFG2	41	1	0	2.4	0.0	60	0	0	0.0	0.0
6.	Fumonisin	20	7	0	35.0	0.0	10	0	0	0.0	0.0
7.	Fumonisin B1	113	26	0	23.0	0.0	60	0	0	0.0	0.0
8.	Fumonisin B2	113	29	0	25.7	0.0	60	0	0	0.0	0.0
9.	FumonisinB3	53	2	0	3.8	0.0	60	0	0	0.0	0.0
10.	FumonisinB4	12	1	0	8.3	0.0					
11.	Ochratoxin	38	24	14	63.2	36.8	10	2	0	20.0	0.0
12.	Ochratoxin A	53	10	0	18.9	0.0	68	1	0	1.5	0.0
13.	Ochratoxin B	41	21	0	51.2	0.0					
14.	Sterigmatocystin	53	8	0	15.1	0.0					
15.	T-2	24	3	0	12.5	0.0	60	0	0	0.0	0.0
16.	HT-2 Toxin	41	7	0	17.1	0.0					
17.	ZEN	89	44	1	49.4	1.1	60	0	0	0.0	0.0
18.	α-ZEA	42	41	0	97.6	0.0					
19.	β-ZEA	41	41	0	100.0	0.0					
20.	DON	101	19	0	18.8	0.0	60	0	0	0.0	0.0
21.	DON-3G	24	3	0	12.5	0.0					
22.	15-Acetyl-deoxynivalenol	77	4	0	5.2	0.0					
23.	3-Acetyl-deoxynivalenol	41	9	0	22.0	0.0					
24.	Nivalenol	53	1	0	1.9	0.0					
25.	Citrinin	53	42	0	79.2	0.0					
26.	DAS	60	13	0	21.7	0.0					
27.	Kojic Acid	53	44	0	83.0	0.0					
28.	Cyclopiazonic Acid	41	6	0	14.6	0.0					
29.	Tenuazonic copper acid	53	5	0	9.4	0.0					
30.	FUS-X	36	3	0	8.3	0.0					
31.	Alternariolmethylether	41	41	0	100.0	0.0	8	1	0	12.5	0.0
32.	Averufin	12	3	0	25.0	0.0					
33.	3-Nitropropionic acid	12	2	0	16.7	0.0					
34.	Malformin C	12	1	0	8.3	0.0					
35.	Moniliformin	12	5	0	41.7	0.0					
36.	Culmorin	12	1	0	8.3	0.0					
37.	Equisetin	12	2	0	16.7	0.0					
38.	Macrosporin	12	2	0	16.7	0.0					
39.	Festuclavin	12	1	0	8.3	0.0					
40.	Quinolactacin A	12	6	0	50.0	0.0	8	2	0	25.0	0.0
41.	Dihydroxymellein	12	1	0	8.3	0.0					
42.	Flavoglaucin	12	7	0	58.3	0.0	8	1	0	12.5	0.0
43.	Curvularin	12	1	0	8.3	0.0					
44.	Berkedrimane B	12	2	0	16.7	0.0					
45.	Purpuride	12	1	0	8.3	0.0					
46.	Methylsulochrin						8	1	0	12.5	0.0
47.	Integracin A						8	1	0	12.5	0.0
48.	Integracin B						8	1	0	12.5	0.0
49.	Methylfunicone						8	1	0	12.5	0.0
50.	HCN	423	257	169	60.8	40.0	20	19	6	95.0	30.0

	KEY	
	Total Number analysed	TA
	Total Number Contaminated	TC
	Total Number above ML	TU
	% Contaminated	PC
	% Above ML	PA

	Summary	
	Number of mycotoxins analysed	49

APPENDIX II

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