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Review: Toxicity Volatiles of Dried Techniques for Detoxification in Cassava Leave *(Manihot esculenta Crantz)*

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Abstract— *Cassava (Manihot esculenta Crantz) contains 310 mg/kg.dw of toxicity, making it extremely toxic to ruminants. The major goal of the research project is to determine the best approach for managing or decreasing the toxicity contents of cassava leaves using a drying method in order to use cassava leaves for ruminant feedstuffs.Based on the previous results, it was discovered that premium hydrogen cyanide (HCN) degradation of cassava leaves was achieved using two different drying procedures (natural drying and oven drying). The first strategy, based on natural drying for 2-3 days, degraded 95.9%; 8 days, 93.2%; 2-3 days, 94%; and 1-2 days, 80% (80-90 °C; 24h, degraded 38.9%), (50 °C; 24h, degraded 31.5%), (60 °C; 20h, degraded 68-76% (leaf) or 24-36% (foliage), (45 °C, 60 ^{0}C, and 75 ^{0}C; 20h, degraded 34%, 53.46%, and 9.35%. As a result, dried at (75°C; 33h and 75°C; 50h) deteriorated to between 0.05 mg kg-1 (99.95%) and 0 mg kg-1 (99.95%), (100% removed). The greatest results were found on dried HCN bound 94-95.9% by SND for 2-3d and OVD for 2-3days HCN bound 94-95.9% by OVD (75 °C; 50h), deteriorated 100%. The cassava leaves drying process by sun-drying alone offers various operational benefits and requires less time to manage since manufacturing and providing ruminant feedstuffs. As a result, because sun-drying (24-36 hours) is particularly practical for small-scale farming, concentration feeding innovators should adopt it. Separately, medium and large-scale farming should use an oven at (75 °C; 33h) to remove 99.95 % of HCN due to its economic efficacy. In particularly, cassava plays an essential role in ruminant farming. Thus, minimum toxicity in cassava leaves could improve the nutritional content in feedstuffs.*

Keywords— *Cassava (Manihot esculenta Crantz), hydrogen cyanide, drying.*

I. INTRODUCTION

Cassava (Manihot esculenta Crantz) is a vital carbohydrate source for millions of people in tropical areas (Nambisan, 1994), and it is one of the most important upland crops in Cambodia (Sopheap *et al.*, 2011), where it has served as a mechanism for improving livelihoods and national income levels, and it is the second largest crop production after rice (MAFF, 2018). The majority of cassava production has been concentrated on the roots, which are utilized in animal feed, ethanol, and other industrial products (Kuakoon and Tanticharoen, 2011).

So, over 2,000 plants produced cyanogenic glucosides, which were broken down using the enzyme linamarinse as a catalyst to produce cyanohydrine and glucose, and then the cyanohydrin hydrolyzed automatically above pH 5 to release hydrogen cyanide gas and a ketone in a second reaction (Howard *et al.*, 2010). Cyanogenic glycoside was discovered in large quantities in the biter leaves of cassava (King and Bradbury, 1995). It had 310 mg/kg in the leaf and 2,450 mg/kg in the tuber (Nartey, 1980). If an animal ate 5-35 mg/kg of HCN per 10 kg of body weight, it would die (Anna et al., 2010). On the other hand, cassava leaf contains phytate and polyphenols, which have antioxidant characteristics and can impede digestion and nutritional intake (Julie *et al.*, 2009). Furthermore, cyanide in foliar cassava might have negative consequences for users, including: 1) Symptoms of headache, dizziness, vomiting, nausea, stomach pains, diarrhea, or death if too much is consumed (Mlingi *at al.*, 1992). 2) Thyroid gland enlargement owing to iodine shortage (Delange et al., 1994). 3) Assist in the development of tropical ataxic neuropathy (TAN), fantasy, loss of hand sensation, loss of vision, hearing, and weakness (Osuntokun, 1994). 4) Contracting konzo illnesses and remaining paralyzed in the legs (Howlett *et al.*, 1990). Reduce cyanogen by adopting conventional procedures such as drying, boiling, parboiling and drying, steaming, baking, frying, and flour preparation to ensure that using leafy cassava has no negative influence on consumers (Kobalwila *et al.*, 2005). This approach can minimize cyanogenic by up to 98%. If a cassava product has more than 250 g CN equivalence/g, achieving safe levels of 10 g/g is a major challenge for ruminants (Milena *et al.*, 2013; Bala, 2010). Farmers have recently harvested solely cassava roots, discarding the foliage. Cassava leaves are generally good quality feed for ruminants, especially during the dry season. The amount of nutrition in cassava leaves varies depending on the variety of cassava, the age of the plant, and the size of the leaves and stems (Cited from Julie *et al.*, 2009). It had 19.5 % crude protein and 40 grams of tannin per kilogram of dry matter (Granum *et al.*, 2007). It's also high in protein, minerals, vitamins B1, B2, and C, as well as carotenoids (Adewusi and Bradbury, 1993).

The dry matter yield of cassava leaves is 7.86-10.90 t/ha (Chalaem, 2017). Farmers would have more opportunities to benefit from gathering foliar cassava for ruminant fodder if this scenario existed. As a result, it's critical to encourage small and large-scale farmers to prioritize the use of this valuable crop for ruminants. The case study will investigate the “Effect of drying processing

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II. Cyanide Metabolism in Cassava

Cyanoalanine synthase activity can be found in all plants. When the metabolism of cyanide in cassava was studied more thoroughly, it was shown that all tissues have the potential to metabolize cyanide. All tissues exhibit high levels of cyanoaline synthase activity, integrate free cyanide into C4 molecule (presumably via cyanoaline synthase), and have rhodanses activity catalyzing the production of thiocynanate from CN^- and S_2O_{32}- (Blumenthal *et al.*, 1968). Linamarin is synthesized and accumulates in the vacuole, as previously stated. The linamarin is transformed to cyanide by the linamarase and hydroxynitrile lyase (HNL) found in the cell wall once the cell is broken. Cyanide translocates and tissue-specific variations in cyanide metabolism have recently been poorly defined, making it difficult to simulate cynogen metabolism at the entire plant level.

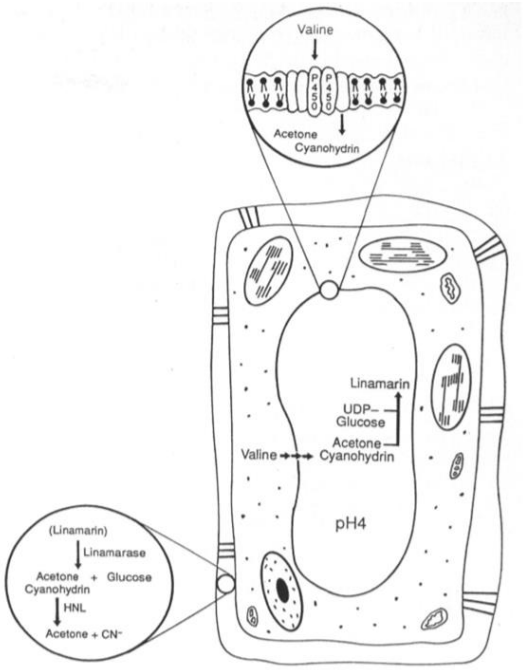

Fig. 1. Cyanide metabolism in cassava
Source: Jennifer et al. (1995)

III. Toxicity Contents Accumulation in Cassava Leaf

Formation of hydrogen cyanide in cassava leaves. The cyanogenic glucosides linamarin and lotaustralin are found in abundance in cassava. Figure 2, respectively. It's made up of the amino acids valine and isoleucine, which act as antecedents (Bokanga, 1994). In cassava, cyanogenic glucosides are biosynthesized in the leaves, translocated, and stored in plant tissues in varying levels (Koch *et al.*, 1992). Cassava leaves, especially petioles, have a high concentration of cyanogenic glucosides, which is likely 5 to 20 times higher than the concentration in the root, and they are largely contained inside the vacuoles cells (Bokanga, 1994). Cyanogenic glucosides cannot break down when the cells are intact. Enzyme and cyanogenic glucosides come into contact as a result of animal chewing or technical processing, resulting in the synthesis of HCN. When the pH is more than 5, hydrocynitrile spontaneously degrades into a keton molecule and HCN (Bokanga, 1994). As a result, when the pH is less than 5, the dissociation process is directly catalyzed by a -hydroxynitrile lyase, resulting in the formation of a keton molecule and HCN. Sugar and -hydrocynitrile are formed when linamarin and lotaustralin are hydrolyzed.

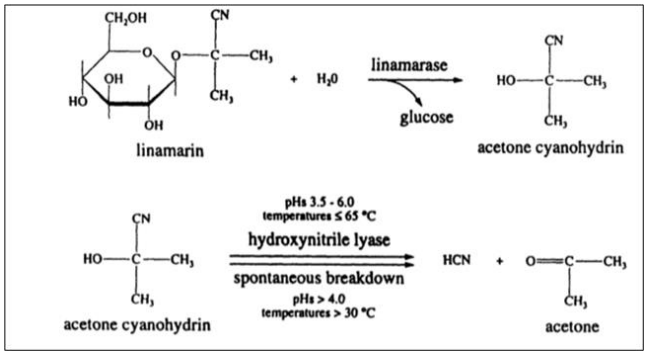

Fig. 2. Cyanogenesis from lanamarin
Source: Jennifer et al. (1995)

IV. Distribution of Cyanoglucosides in Cassava

The cyanogenic glucoside linamarin is found in 95% of cassava tissue and lotaustralin is found in 5% of cassava tissue (Nartey, 1968). Linamarin is found in higher concentrations in the cortex of leaves, stems, and roots (>400 mg/kg HCN eq.) than in the parenchyma root (100 mg/kg). When cassava leaves get older, amount of cyanide in them drops to 50%, compared to 70% in young leaves (Nambisan and Sundaresan, 1994). Bitter cassava has cyanogenic glucoside levels ranging from 320-1,100 g/g, according to previous study (Sinha and Nair, 1968).

V. Metabolism of Cyanoglucosides in Cassava

The aminoacids valine and isoleucine are the most important in the production of linamarin and lotaustrlin, and the basic pathway involves a mutlienzyme complex in the microsomes sequentially converting the aminoacids to N-hydroxyaminoacid, oxime, and hydroxynitrile. The following biochemical and physiological processes may influence the buildup of cyanoglucosides in cassava: 1) linamarin biosynthesis, 2) linamarin catabolism and turnover, and 3) linamarin transfer from leaves to roots. The diversity in cultivars reflects the success of these procedures' linamarin levels (Nartey, 1969).

VI. Elimination of Cassava Cyanogen by Processing

A. Drying Methods

The cassava drying is used two kinds of methods to remove hydrogen cyanide (HCN) from cassava leaves: mechanical drying such as natural drying (sun-drying) and oven-drying (table 1, 2, 3, 4, 5), according to the Ayodeji (2005; 2005[c]); Padmaja (1989); Gomex and Valdivieson (1985[a]); Nambisan and Sundaresan (1985). Endogenous linamarase regulates the removal of cyanogenic glucosides throughout the drying process, and is hence responsible for the accumulation of cyanohydrin and free cyanide in dried

cassava (Julie, 2009). The report of the Ayodeji (2005) has been elucidated the effect to decline toxity from the cassava leaves by sun-drying (SND) for 24-36 h was effected between 1.7-4.1 mg HCN/100g or total average retained 4.1% or 95.9% removed, while oven drying (OVD) 80-90^{0}C for 24 h was least efficient (i.e. 24.9-39.3 mg HCN/100g or 61.1 % retained or 38.9 % removed, table 1) and it has 41 HCN mg HCN/kg FW and retained 6.8 % or 93.2% removed by SND for 8 days, while OVD 50 ^{0}C was least efficient (i.e. 293 HCN mg HCN/kg FW or 64.9% retained or 35.1% removed, table 2, part c). During sun-drying for 24-36h, 82 to 94% of the initial cyanide was eliminated; while 68-76% of cyanide was removed during oven drying at 60 ^{0}C for 20h (table 3, a.1). In the sun drying foliage is 62-77% existed as bound cyanide and 24-36% existed in bound form in oven-dried foliage (table 3, a.2) was reported by Gomex and Valdivieson (1985[a]).

TABLE 1: Hydrocyanic acid content (mg/100g) in different processing in cassava leaves (Sun-drying 24-36h and Oven drying 80-90 ^{0}C; 24h)

Processing variable	Cassava varieties				
	MS-6	TMS-30555	TMS-30572	Local	HCN retained (%)
SND	1.8(3)	1.7(4)	1.8(3)	4.1(6)	4.1±1.1
OVD	41.5(74)	24.9(62)	24.9(46)	39.3(65)	61.6±11
STM (30 min)	27.3(48)	18.4(46)	36.7(68)	45.2(75)	59±14.3
SHD (5variance pieces)	26.4(47)	19.6(49)	23.7(44)	30.8(51)	47.5±3.0
STP	35.7(63)	27.8(69)	36.7(68)	46.1(76)	69.1±5.3
STM+SND	37(66)	15.3(38)	16.6(31)	26.1(43)	44.3±15.0
SHD+SUN	1.6(3)	1.7(4)	1.7(3)	2.9(5)	3.7±0.1
Fresh leaf	56.5(100)	40.2(100)	54.1(100)	60.6(100)	(100)

SND=Sun-drying, OVD=Oven drying, STM=Steaming, SHD=Shredding, STP=Steeping, % HCN retained

Source: Ayodeji (2005)

Separately, reported by Nambisan and Sundaresan (1985), HCN retained 20-50% or 50-80% removed by using SND for 24-48 h; while 30-50% of cyanide retained or 50-70% was removed during oven drying at 40 ^{0}C for 24 h (table 4). Additionally, The fresh cassava mature leaves contents HCH ranging from 900-1,293 total of HCN mg HCN/kg FW was reported by Bokanga (1994[a]), table 2 and 436-736 mg/kg (dwb) of total cyanide and 254-443 mg/kg (dwb) free cyanide (Padmaja, 1989), table 5. Rapid dehydration occurring during oven drying on the fresh leaves and foliage mature at at (80-90^{0}C; 24h, degraded 38.9%, table 1), (50 ^{0}C; 24h, degraded 31.5%, table 2), (60 ^{0}C; 20h, degraded 68-76% (leaf) or 24-36% (foliage) table 3, a.1 and a.2), (45 ^{0}C, 60 ^{0}C and 75 ^{0}C; 20h, degraded 34%, 53.46% and 9.35%, table 5) were responsible for the higher retention of bound cyanide in high temperature. Separately, dried (40 ^{0}C; 24h, degraded 80%, table 4) much reduced toxity content, respectively. As a result, linamarase activity is suppressed at temperatures above 550°C, and linamarin begins to build in dried cassava (Mlingi and Bainbridge 1994). The drying temperature of cassava leaves was found to have a substantial impact on cyanide reduction. When leaves were dried at temperatures over 60°C, cyanide retention was found to be low (table 1, 3, 5).

The processing by sun-drying is very efficient for removal of cyanide hydrogen glucoside (CHG) because of the prolonged contact time (Padmaja and Keith, 2009). Additionally, it also has some factors to impact of cyanide degradation such timing period, duration of sun-drying, and cultivars. Actually, duration of SND for 2-3d, degraded 95.9%, table 1; 8d, degraded 93.2%, table 2; 2-3d, degraded 94%, table 3 and 1-2d; degraded 80%, table 4 were reported by Ayodeji (2005; 2005[c]); Nambisan (1994[a]); Bokanga (1994[b]); Gomex and Valdivieson (1985[a]); Nambisan and Sundaresan (1985). Furthermore, wilting during sun-drying facilitate linamarase union, bringing hydrolysis of the volatile hydrogen cyanide. Ambient drying between 30-50 ^{0}C, the linamarin breakdown to acetone cyanohydrin catalyze by linamarase and its spontaneous decomposition to HCN (Bokanga, 1994; Nambisan, 1994). Cyanogenic glucosides breakdown during sun-drying depends on enzymatic hydrolysis and on gradual leaf cell disintegration (Mlingi and Bainbridge 1994).

TABLE 2: Declining of cyanogen contents from cassava leaves by using different processing techniques (Sun-drying 8 days and Oven drying 50 ^{0}C; 24h)

	Cyanide retention (%)	Total HCN mg HCN/kg FW
Fresh leaves [a]	100	420
Chopped and boiled (15min)	14.5	60
Crushed and boiled	3	15
Chopped and dried (70 ^{0}C)	70	300
Fresh leaves [b]	100	900.3-1293.6
Pounding (15min)	63-73	298.7-447.9
Boiling pounded leaves (15min)	0.3-1.2	4-11.1
Fresh leaves [c]	100	606
Sun-drying	6.8	41
Oven-drying	64.9	393
Steaming	74.6	452
Shredding	50.8	308
Steeping	76.1	461
Steaming + Sun-drying	43.1	261
Shredding + Sun-drying	4.8	29

Source: Nambisan (1994 [a]); Bokanga (1994[b]); Ayodeji (2005[c])

TABLE 3: Cyanide reduction during sun-drying (24-36h) and Oven drying (60 ^{0}C; 20h) of cassava foliage

Cassava cultivar (a.1)	**Percentage of elimination of cyanide**	
	Sun-drying	Oven drying
M Col 22	82±60	69±13
M Col 1684	87±70	76±10
CM 342-170	88±50	68±40
M Col 113	94±20	74±40
(a.2)	**Residual free cyanide(% total)**	
	Sun-dried foliage	Oven-dried foliage
M Col 22	77±70	26±90
M Col 1684	75±90	36±17
CM 342-170	66±70	24±11
M Col 113	62±22	25±11
(b.1)	**Wilting, drying (^{0}C 60)**	
	Total	Detoxification
M4	55.3±2.50	33.34±2.10
H1687	60.10±3.40	39.80±1.80
H165	83.32±7.30	53.16±3.40
H226	80.25±2.80	46.65±4.60
H2304	58.30±3.50	26.65±1.20
(b.2)	**Wilting, chopping, drying (^{0}C 60)**	
	Total	Detoxification
M4	98.95±6.50	39.90±4.30
H1687	106.65±9.30	46.67±20
H165	98.60±3.40	60.48±1.90
H226	93.32±6.80	66.50±3.40
H2304	133.30±12.10	70.0±2.80

Source: Gomex and Valdivieson (1985[a]); Padmaja (1989[b])

TABLE 4 : removal of cyanide from cassava during processing (Sun-drying 24-48h and Oven drying 40 ^{0}C; 24h)

Method of processing	**Percentage**	**Mechanism**
	Cyanide retained	removal
Boiling	20-50	Leaching
Blanching and drying	50	Leaching
Baking, frying, steaming	80	Thermal degradation
Sun-drying	20-50	Enzyme action
Oven	30-50	"
Crushing and sun-drying	<5	Disintegration and Enzyme action
Grating/fermentation, dewatering, drying	<2	"

Source: Nambisan and Sundaresan (1985)

TABLE 5: Effect of drying temperature between (45 ^{0}C, 60 ^{0}C, 75 ^{0}C; 20h) on the cyanide content of cassava leaves

Variety	**Cyanide content (mg/kg dry matter)**			
	Fresh leaves	45 ^{0}C	60 ^{0}C	75 ^{0}C
M4				
Total	436.52±20.1	86.75±6.5	77.34±3.4	138.00±7.4
Free	254.64±6.5	66.60±3.4	50.60±2.8	90.65±3.5
H1687				
Total	542.26±32.5	113.32±9.8	76.00±6.5	141.33±8.1
Free	299.18±9.8	66.40±7.6	46.54±2.4	102.00±6.5
H165				
Total	729.24±19.6	186.65±11.1	153.32±9.7	233.31±13.2
Free	373.97±12.5	86.56±5.4	60.00±3.9	113.3±4.3
H226				
Total	736.22±12.5	166.66±13.2	113.32±10	233.35±10.5
Free	370.52±7.3	100.6±6.1	59.94±4.6	140±5.1
H2304				
Total	709.14±17.6	183.98±9.8	150.65±11.3	253.3±15.1
Detoxification	443.21±8.8	99.98±2.8	80.00±7.8	146.65±2.9

Source: Padmaja (1989)

Accumulated research results base, it has been elucidated that twice variances techniques followed by drying (natural drying and oven drying) were premium HCN degradation of cassava leaves. First technique, based on natural drying 2-3d, degraded 95.9%, table 1; 8d, degraded 93.2%, table 2; 2-3d, degraded 94%, table 3 and 1-2d; degraded 80%, table 4. Dried by OVD at (80-90^{0}C; 24h, degraded 38.9%, table 1), (50 ^{0}C; 24h, degraded 31.5%, table 2), (60 ^{0}C; 20h, degraded 68-76% (leaf) or 24-36% (foliage) table 3, a.1 and a.2), (45 ^{0}C, 60 ^{0}C and 75 ^{0}C; 20h, degraded 34%, 53.46% and 9.35%, table 5. Therefore, dried at (75 0C; 33h and 75 0C; 50h) degraded between 0.05 mg kg-1 (99.95% removed) and 0 mg kg-1 (100% removed), table 6, figure 3, respectively. The best result finding on dried by SND for 2-3d HCN bounded 94-95.9% and OVD for (75 0C; 50h), degraded 100%.

VII. Discussion

Graphic 1: Accumulated of Previous Research Finding on Traditional Processing Techniques

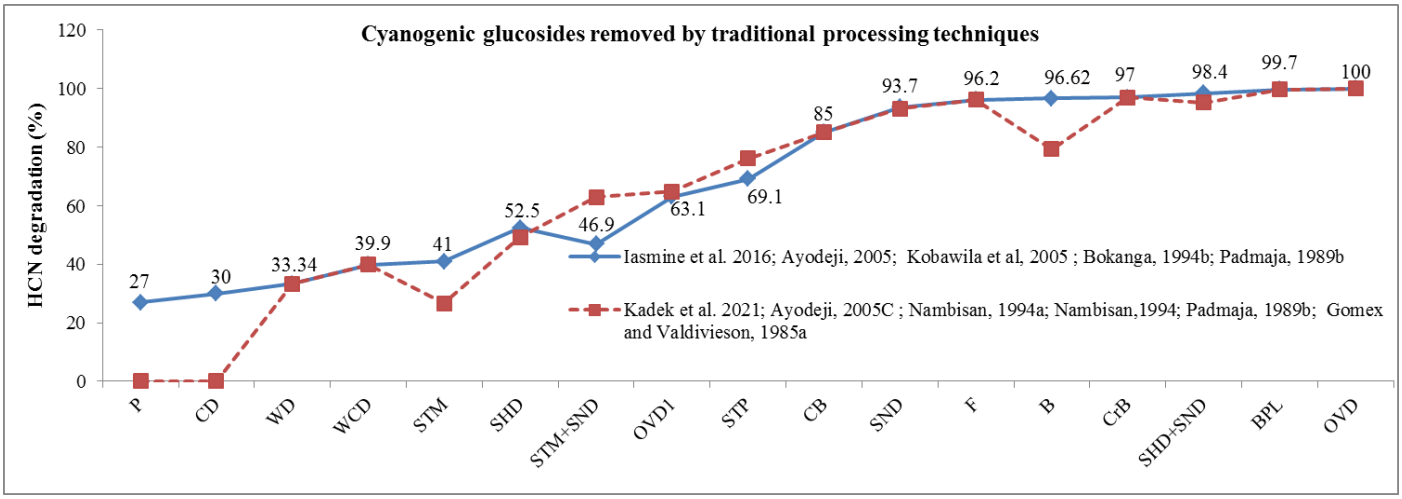

Fig. 3. Declining of HCN from cassava leaves
SND=sun-drying,CD=chopped+drying, WD=wilting+drying, OVD=oven-drying, STM=steaming, SHD=shredded sun-drying, P=pounding, STM+SND=steam + sundry, STP=steeping, SHD+SND=shredded + sundry, CB=chopped & boiled, CrB=Crush boiling, BPL=boiling pounding leaf, F=fermentation, and WCD=wilting+chopped+dring, B=Boiling

The previous research finding results to remove the cyannogenic glucosides (HNG) from cassava leaves with traditional processing techniques have seven premium effected processing techniques; SND, CrB, BPL, SHD+SND, and F, HNG was removed between 93.7-99.9% (Nambisan 1994; Bokanga, 1994b; Ayodeji 2005; Ayodeji 2005c; Kobawila *et al.*, 2005) have been reported. Separately, boiling for 15 minutes the cyanide removed 96.62 (Kadek *et al.*, 2021). Furthermore, according to the Iasmine et al. (2016) found that Oven drying has effectively removed cyanide between 99.95-100%.

VIII. Conclusion

According to the accumulated research finding results; cassava leaves drying was used two kinds of methods to remove hydrogen cyanide (HCN) from cassava leaves: mechanical drying such as natural drying (sun-drying) and oven-drying. First technique, based on natural drying for 1-8 days, it was affected to decrease HCN between 80-95.9%. Enzymatic hydrolysis and progressive leave cell disintegration are required for cyanogenic glycosides to degrade during sun drying. Meanwhile, cassava cultivar, time period and dried times are main indicator that makes HCN remained inequitable. Separately, dried by OVD at (40-90 ^{0}C; 20-50 h) eliminated between 9.35-100% of HCN. The temperature and dried times are distribution factor to remained in different level of HCN. The best result of dried by SND for 2-3d HCN bounded 94-95.9% and OVD dried at (75 0C; 50h) degraded 100%, and (75 0C; 33h) degraded 99.95%. Therefore, concentration feeding developer should use sun-drying (24-36h); due to it is so convenience for small-scale farming practicing. Separately, medium and large-scale farming should use oven at (75 0C; 33h) declined 99.95% of HCN due to it is economy effectiveness. In particularly, cassava plays an essential role in ruminant farming. Thus, minimum toxicity in cassava leaves could improve the nutritional content in feedstuffs.

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