Cyanide detoxification methods in food: A review

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Abstract. Cyanide is a toxic substance found in several tubers such as cassava (Manihot esculenta), wild yam (Dioscorea hispida Dennst), some cerealia and legumes. In the plants, it can be in the form of cyanogenic glycosides, acetone cyanohydrin, and hydrogen cyanide (HCN). Cyanogenic glycosides such as linamarin and lotaustralin belong to the product of secondary metabolism. The characteristic of cyanogenic glycosides is intermediately polar, water-soluble, and often accumulated in the vacuoles of plant cells. Acetone cyanohydrins are intermediates product from cyanogenic glycosides and cyanide acid in plant tissues. HCN, hydrogen cyanide, is a volatile and water-soluble compound. The toxic effect of cyanide in humans is inactivation of cytochrome oxidase, respiratory disorders, sore throat, dizziness, limpness, convulsions, and lethal effects. Cyanide can be removed by several processes before consumption. Methods such as peeling, washing, heating, drying, fermenting and chemical treatment are used to remove or reduce cyanide. The treatment can damage the structure of the cell and hydrolyzed the cyanogenic glycosides to acetone cyanohydrin and glucose by endogenous enzyme like linamarase. A second enzyme, hydroxynitril lyase, can dissociate acetone cyanohydrins to HCN, ketone and aldehyde compound. The maximum safe level for total cyanide in food is 10 ppm. This review aims at updating the available knowledge on the various detoxification cyanide in food.

1. Introduction
Cyanide is a highly toxic anion [1]. The toxic compound found in several plants like cassava and wild yam. Cyanide is dangerous for humans and animals. Cyanide includes cyanogenic gluicosides, acetone cyanohydrin, and free HCN [2]. The limit doses cyanide to be used in food products was 10 ppm [3], in which its doses of 112-140 ppm can cause death in 0.5-1 hour, 140 ppm within 30 minutes, 188 ppm within 10 minutes, and 282 ppm was extremely dangerous [4]. Cyanide residue consumption cause neurological disorder, paralysis, breath, hypotension, tachycardia, headache, stomachache, nausea, diarrhea, dizziness, mental confusion, and seizures [5,6,7]. Moreover, cyanide's continuous consumption in low doses brings up effects such as goiter, stunting, and neurological disease [2]. Therefore, the detoxification process must be done to eliminate toxic compounds.

Cyanide detoxification can increase shelf life, value, and utilize the material. Several previous studies were carried out to determine which detoxification method effectively eliminated and reduced toxic compounds like cyanide. The detoxification method was done through different processes, along with
different conditions and materials. The traditional detoxification, which was mostly done by the community, was smearing with ash and salt [8], sun drying, and soaking in running water [9]. The other researchers also conducted the cyanide detoxification through the heat process [10], the fermentation by utilizing the microbes [11], enzymatic [10], and the use of equipment like ultrasound [10]. Sun-drying eliminates more cyanide than oven drying because of the prolonged contact time between linamarase and the glucosides in sun drying. Soaking followed by boiling is better than soaking or boiling alone in removing cyanide [12].

The detoxification process can damage the cell wall causes contact between cyanogenic glycosides and beta-glucosidase enzyme. Therefore, cyanogenic glycosides convert into glucose and cyanoxydrin [13]. Cyanohydrin is stable under acidic conditions but can decompose at pH>5.0 or catalyzed by lyase enzyme [14] into HCN. The boiling point of free HCN is 26°C causing HCN to evaporate easily [15]. This paper is aimed to review the method of detoxification of cyanide in plants.

2. Discussion

2.1. Cyanide

Cyanide is a chemical compound whose group of C ≡ N, that is attached to the hydrogen atom and the carbon atom is attached to the nitrogen atom [16]. In general, cyanide is available in cyanogenic glycosides, acetone cyanoxydrin, and free HCN. Commonly, cyanogenic glycosides are found in the plant roots in which will be distributed to the other parts such as leaves, fruit, or tubers [6]. The types of cyanogenic glycosides contained in plants also differ, like linamarin and lotaustralin in wild yam and cassava tubers, as well as dhurrin in a bamboo shoot [17].

Cyanide compounds also affected the health of the human body negatively. The food products which did not have an effective detoxification process, have cyanide left the form of linamarin, acetone cyanoxydrin, or free HCN in the material [18]. Some un-effective detoxifications are leaching and steaming. The methods have more than 10 ppm residues cyanide [18]. Cyanogenic glycoside like linamarin compound was hydrolyzed into acetone cyanoxydrin by microorganisms found in the intestine. The acetone cyanoxydrin was degraded spontaneously in the small intestine in which it had alkaline pH conditions. This degradation formed HCN, which bound to methemoglobin. After that, it was converted into isothiocyanate by the rhodase enzyme found in the liver, and it obstructed the mitochondrial oxidation [5] that causes histotoxic anoxia [19].

Cyanide found in food plant, such as cassava [5], bamboo shoot [17] and wild yam [20]. Bamboo shoot contained 245 mg/100 gram cyanide, the cyanide content found in wild yam tuber varied widely, 469.5 ppm in fresh wild yam tuber, 13.67–60.88 ppm in flour [17], and 50-400 ppm in the wild yam tuber and 19.95 ppm in wild yam flour [20].

2.2. Detoxification cyanide

Detoxification is the process of removing toxic compounds from the material [21]. Cyanide is one of the unwanted poisons that must be removed from the material. The detoxification process is performed to make material safe for consumption [22].

The principle of detoxification is to reduce cyanogenic glycosides such as linamarin and lotaustralin, and dhurrin in the material. The cell wall can damage and open vacuoles. After that, cyanogenic glycosides can contact with enzymes and form cyanoxydrin. Cyanohydrin will degrade spontaneously to HCN [23]. The boiling point of free HCN is 26°C causing HCN to evaporate easily [24]. In general, cyanide changed can be seen in Figure 1.
2.3. Detoxification methods

Detoxification methods that have been researched varied widely in different regions. There are many different methods of detoxifying cyanide before it is made into a consumable product, including the use of heat, enzymatic, chemicals, and ultrasound [8][10][11][17]. The decrease of cyanide in the material after the detoxification process varies depending on the process and the conditions of the raw material. Cyanide in cassava was detoxified by pulverizing for 15 minutes and followed with a boiling process for 10-120 minutes [5]. Other cyanide detoxification methods are heat treatment, enzyme assistance, microorganism assistance from the fermentation process, use of chemicals such as sodium bicarbonate, and tools such as ultrasound [10].

2.3.1. Heat treatment

Cyanide detoxification can be performed using heat treatment. Detoxification of cyanide was performed by [10] on cassava leaves by using heat at a temperature of 25°C-85°C for 36 hours. The crushed cassava leaves were mixed with water and homogenized. The sample was then heated at 25°C, 35°C, 55°C, 75°C, and 85°C for 36 hours. The results showed that during the heat incubation process, the cyanide content decreased by 90%. It decreased from 534 ppm to 50 ppm after incubation for 36 hours. That is caused by linamarase enzyme activity which worked optimally at 55°C. Therefore, cyanogenic glycosides contact with enzyme and were converted into acetone cyanohydrin [26]. Second, there was the HNL (hydroxy nitrile lyase) enzyme, which was stable at 60°C. Third, acetone cyanohydrin's spontaneous decomposition into HCN and acetone occurred at temperatures above 30°C. Fourth, HCN evaporation occurred because the boiling point of HCN was 26°C [10].

Another research was conducted by [17] by detoxifying through the curing process and limited heating. The curing treatment for 12 hours at a temperature of 50°C left a cyanide content of up to 13.67 ppm with cyanide content in fresh wild yam tubers of 469.50 ppm. The decrease of cyanide reached 97.09% because the curing process optimized the glucosidase enzyme action, an endogenous enzyme that catalyzed the breakdown of cyanogenic glycoside compounds into free cyanide. The hydrolysis process done by β-glucosidase on cyanogenic glucosides produced sugar and hydroxy nitrile. The hydroxy nitrile was then broken down enzymatically into HCN and a carbonyl mixture. Free HCN can dissolve with water and evaporate at 26°C [18].

Another research conducted cyanide detoxification of wild yam tubers using leaching and steaming techniques. The best treatment in removing cyanide was in the leaching process with a water flow rate of 5.00x10-5 m³.s⁻¹ for 60 minutes, followed by steaming for 60 minutes. The remaining cyanide was 29.9 ppm, with cyanide content in fresh wild yam tubers of 84.26 ppm, where the decrease was 50.55% [18].

2.3.2. Enzymatic

The cyanide can be detoxed by using the enzyme. In the research conducted by [10], detoxification was carried out using cellulases, hemicellulases, xylanases, and beta-glucanase. These enzymes were produced from Trichoderma reesei 6200 international unit (IU)/mL. Materials were treated with an

![Diagram](image_url)
enzyme concentration of 8% of their dry weight by preparing a 0.32% solution of the liquid enzyme in 0.1 M Na$_3$C$_6$H$_5$O$_7$ buffer at pH 5. The results showed a decrease in linamarin content reached 90.3% after the incubation process at 50°C using xylanase and cellulase enzymes. It happened because of cell damage and contact with linamarin and linamarase increased. Linamarin compounds contact with the linamarase enzyme caused the formation of acetone cyanohydrin. Therefore, linamarin levels decreased. The use of enzymes caused a decrease in cyanide from 559 ppm to 118 ppm. The cyanohydrin acetone was relatively stable under acidic conditions but decomposed spontaneously at pH>5.0. The contact between substrate and enzyme will be faster because of disrupted the bondage between cellulose, lignin, hemicellulose and xylan by enzyme cellulases, hemicellulases, xylanases, and beta-glucanase [10].

2.3.3. Fermentation

One of the detoxification methods is fermentation. The use of fermentation is considered effective because of the help of microorganisms. These microorganisms can cause cell wall damage, leading to a decrease in cyanide. Research conducted by [11] on sorghum, which had a cyanogenic glycoside compound, namely dhurrin as much as 30% of the dry weight. In young plants, dhurrin is usually found in the vacuole of epidermal cells, while the enzyme that hydrolyzes dhurrin is found in mesophyll cells. Dhurrin was hydrolyzed by the endogenous enzyme β-glucosidase (dhurrinase), which then produced p-hydroxy mandelonitrile, which was then separated to free HCN and p-hydroxybenzaldehyde. Hydrolysis of dhurrin by β-glucosidase (dhurrinase) enzymes and production of HCN can be seen in Figure 2.

![Figure 2](image-url)

**Figure 2.** Hydrolysis of dhurrin by β-glucosidase (dhurrinase) enzymes and production of HCN [11].

Another research on cyanide detoxification was on sorghum using the microorganism *S. cerevisiae* NCIM 3156. Sorghum solution containing 14.7% total sugar and 47 ppm cyanide was added to 250 ml of Erlenmeyer then given H$_2$SO$_4$ to pH 4.5. *S. cerevisiae* NCIM 3186 was inoculated as much as 10% (v/v), followed by incubation at 30°C for 75 hours. Every 10 hours analysis of the total residual sugar, ethanol, and cyanide was analyzed. The results obtained were a decrease in cyanide content (84.58%), an increase in ethanol yielded, and fermentation efficiency (91.8%) [27].

Research in improving cyanide detoxification methods to reduce total cyanide in Burundi. The method used was the fermentation process using several strains of microorganisms such as natural culture (spontaneous fermentation), mixed culture, *Lactobacillus Plantarum* V22, *Saccharomyces cerevisiae* (AEB, Brescia, Italy), *Oenococcus oeni*, and *Saccharomyces cerevisiae*. The grated cassava samples were then inoculated with bacterial strains and fermented at temperatures (25, 30, or 35°C) for (24,48, and 72 hours) compared to controls (spontaneous fermentation). The analysis performed included chemical analysis (crude fiber, ash, water), protein (Kjeldahl), tannins (Folin-ciocalteu), cyanide (picrate paper kit). The results obtained no significant differences in the levels of ash, tannins, protein, water, and fiber. The decrease in cyanide levels was significantly different from the microbial strains used. The highest decrease in cyanide levels was found in cassava fermented using *S. cerevisiae* strains for 48 hours at 35°C, a decrease in cyanide of 65.9% [28]. The microorganism will be disrupted.
by plant tissue by producing enzyme so that the contact between the substrate and the enzyme occurs, and the hydrolysis process will be faster [11].

2.3.4. Chemical

Another research regarding detoxification methods that reduced the cyanide content of the material while maintaining the nutrients in them was the addition of sodium bicarbonate. Sodium bicarbonate damaged plant cells and facilitated contact between linamarin and linamarase enzymes. Sodium bicarbonate also increased pH, which resulted in the spontaneous decomposition of cyanohydrin acetone. The detoxification method included crushing 1g of cassava leaf sample, which has been added with 5 ml of 0.4% NaHCO₃ using a homogenizer for 3 minutes and the solution was transferred to 50 ml Erlenmeyer and then stored in a water bath at 55°C for 6 hours. Furthermore, the incubation time effect was determined by incubating the homogenized sample at 25°C for 0.5, 3, and 6 hours. As a control for the effect of NaHCO₃, the leaves were crushed by adding distilled water without the addition of NaHCO₃ and incubated at the same temperature and time. Cyanide levels were known using the picrate paper test method. The results obtained showed that the addition of sodium bicarbonate reduced cyanide by 93% [10].

Previous research by Pramitha and Wulan (2017) has shown that different detoxification methods produced different effectiveness [8]. Detoxification of cyanide of wild yam tubers with a combination of ash soaking and boiling, where the best treatment of wild yam flour was from treatment of 45% husk ash and 30 minutes of boiling. The cyanide content of wild yam flour was 2.70 ppm from the raw material of 628.64 ppm. The decrease in cyanide levels reached 99.57% because cooking dissolved and evaporated HCN compounds and rice husk ash as adsorbents that pulled out cyanide from the material, which then moved through the carbon pores and was absorbed into the inside of the carbon wall resulted in reduced cyanide content [8].

The research by Luthfi et al. (2012) was done by adding an absorbent material like ash and salt to the surface of the wild yam slices [12]. The activated carbon can remove 41.78% HCN, husk ash removed 56.58% HCN and wood ash removed 63.78% HCN. Wood ash absorbent materials were more effective because of compounds containing strong bases, which were Ca and K, whose levels were higher than other types of absorbent materials. At the time of immersion, Ca and K in the absorbent material, apart from reacting with HCN, also reacted with O₂ around the immersion, which caused the formation of salts that were soluble in water HCN was easily dissolved in water [12]. The advantage of chemical treatment is to accelerate the contact between the enzyme and the substrate by damaging plant tissue using chemicals [10]. The reaction of alkaline and HCN can be seen in Figure 3.

$$\text{Ca}^{2+} + \text{O}_2 \rightarrow \text{CaO}$$

$$\text{CaO} + \text{HCN} \rightarrow \text{CaCN} + \text{H}_2\text{O}$$

$$\text{K}^+ + \text{O}_2 \rightarrow \text{K}_2\text{O}$$

$$\text{K}_2\text{O} + \text{HCN} \rightarrow \text{KCN} + \text{H}_2\text{O}$$

Figure 3. The reaction of alkaline and HCN [12].

3. Conclusions

The cyanide detoxification methods were carried out before the material is consumed. The effectiveness of different processing methods in reducing cyanide are different. Detoxification methods that have been performed were the use of heat, enzymatic, chemicals, and ultrasound. Also, the decrease in cyanide in the material after the detoxification process varied, depending on the process and raw material conditions.
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