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ANALYTICAL VALIDATION OF CYANIDE DETECTION LIMITS IN *Gethuk*: IMPLICATIONS FOR FOOD SAFETY AND PUBLIC HEALTH

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ABSTRACT
Gethuk, a traditional Indonesian staple made from cassava, poses potential health risks due to naturally occurring cyanogenic glycosides. While traditional processing is known to reduce toxicity, empirical validation of detection limits in specific food matrices remains critical for ensuring food safety. This study aims to fill the research gap regarding the sensitivity of the complexometric titration method in monitoring cyanide levels in *Gethuk*, specifically evaluating its effectiveness against national safety standards. Qualitative analysis was conducted using the picrate paper test, while quantitative assessment utilised a modified Deniges complexometric titration. The results indicated that cyanide was not detected in any samples, with levels remaining below the method's limit of detection (LoD) of 14.43 mg. However, the high LoD relative to the Indonesian National Standard (SNI) of 1 mg/kg reveals a significant methodological limitation for trace analysis. These findings underscore the urgent need for more sensitive analytical techniques to ensure public health safety. By evaluating food safety through analytical validation, this research directly contributes to Sustainable Development Goal-3: Good Health and Well-being, providing a foundation for improved monitoring of traditional food products to mitigate chronic toxicological risks
Keywords: Cyanide, Complexometric Titration, Limit of Detection, *Gethuk*, Food Safety, SDG-3, Public Health.
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INTRODUCTION

Cyanide is a highly dangerous poison because it can inhibit the body's metabolic processes by blocking oxygen use by cells, causing organ damage and even death.[1,2] This compound is naturally found in various foods such as cassava, almonds, apple seeds, and several other plants that contain cyanogenic glycosides, which can produce hydrogen cyanide when digested. Exposure to cyanide from these foods has the potential to cause poisoning, which can be seen in symptoms such as nausea, dizziness, and even fatal respiratory problems if not treated immediately. Therefore, the presence of cyanide in food is a serious concern for food safety and public health.[3,4] In direct alignment with Sustainable Development Goal-3 (SDG-3) concerning Good Health and Well-being, accurately determining the detection limit of cyanide compounds in traditional Indonesian foods like *gethuk* is imperative to mitigate dietary health risks and safeguard public safety.[5,6] Given the serious health risks posed by cyanide even at low concentrations due to its biochemical toxicity, accurately determining its presence in food products necessitates analytical methods with appropriately sensitive detection limits; thus, understanding and validating the Limit of Detection (LoD) is essential to ensure reliable identification and quantification of cyanide, enabling timely detection and prevention of potential poisoning incidents. A key factor in chemical testing is the Limit of Detection (LoD), which means the smallest amount of a substance that a specific testing method can find or identify. Even though an analyte at the limit of detection can be noticed, this level doesn't guarantee that the measurement is accurate, precise, and reliable enough. Therefore, it is important to determine the level of detection carefully and properly so that the analytical method can be used in the best way possible, especially when dealing with dangerous substances like cyanide. When doing cyanide tests, it's important to find the right level of detection because even tiny amounts of this substance can be very harmful to health. If the level of detection used in an analytical method is too high, it might miss cases of cyanide poisoning early on, which could delay treatment or prevent the discovery of contamination altogether. So, creating and checking analytical methods that can

detect and measure cyanide accurately is really important. It helps make sure our food is safe and also protects the environment from the harmful effects of cyanide.[3,4] Cyanide analysis techniques have changed over time, starting with methods like complexometric titration and spectrophotometry and now including chemical sensors and easy-to-use test kits. Each method has different levels of how well it works, how correct it is, and how consistent the results are. Creating and testing methods that have a low limit of detection is important to meet international safety rules and standards, like those set by the WHO and SNI. Checking the LoD testing also makes sure the method works well for regular checks. Besides the technical parts about how sensitive and accurate an analytical method is, figuring out the Limit of Detection (LOD) in cyanide testing is also important. It helps pick the best testing method that works well with the type of sample being tested. These sample matrices can be very different. They can range from drinking water, which is usually not very deep, to complicated and thick food materials, to industrial products that have many chemicals that might affect the analysis. Each matrix has its own special problems to deal with, like unwanted materials that can create wrong results or hide the cyanide, changes in the sample's physical and chemical makeup that influence the test reaction, and environmental factors that need the method to be adjusted. So, when creating an analytical method, it's important to also do thorough testing to check the limit of detection. This helps make sure the method can find cyanide accurately and in the same way every time, no matter what kind of sample it's being used on. This check involves testing how consistent the results are, how well it works in the presence of other signals, and how effective the method is when dealing with tough sample situations.[7] Ultimately, this process becomes the main foundation in determining the best analytical method that not only meets technical standards but is also relevant and effective for the analytical needs of specific sample types so that the test results can be trusted and used as a basis for decision-making in food safety supervision and environmental protection. The aim of this research is to determine the detection limit (LoD) of cyanide compounds in traditional Indonesian food, *gethuk,* using the complexometric method.

EXPERIMENTAL

Material and Methods

The sample utilised in this research was the traditional food product, *Gethuk*. The chemicals utilised included distilled water, 5% tartaric acid, 8% sodium carbonate (Na2CO3), 2.5% sodium hydroxide (NaOH), ammonium hydroxide (NH4OH), 5% potassium iodide (KI), silver nitrate (AgNO3), sodium chloride (NaCl), potassium chromate (K2CrO4), and potassium thiocyanate (KSCN) used for simulating cyanide concentrations. Instrumentation: Key equipment included Erlenmeyer flasks, picrate paper, standard laboratory glassware, a steam distillation apparatus, and a burette for titration.

Sample Preparation (Qualitative)

A 50 g portion of the mashed *Gethuk* sample was weighed, and KSCN was added at varying concentrations (0.04% to 0.002%) to simulate cyanide presence. The mixture was macerated with 100 mL of distilled water, followed by the addition of 20 mL of 5% tartaric acid.[8,9]

Sample Preparation (Quantitative)

A 20 g portion of the *Gethuk* sample (spiked with KSCN ranging from 0.1% to 0.005%) was macerated for 2 hours and subsequently subjected to steam distillation. The distillate was collected in an Erlenmeyer flask containing 20 mL of 2.5% NaOH solution until a total volume of 100 mL was obtained.[9]

Standardization

The $AgNO_3$ titrant was standardised using a 0.0141 N NaCl standard solution with 5% K_2CrO_4 indicator until a brick-red precipitate was formed.

Detection Method

Qualitative Method (Picrate Paper Test)

The detection was performed using picrate paper wetted with 8% Na_2CO_3. The paper was suspended over the sample in a closed Erlenmeyer flask and heated for approximately 15 minutes. A positive result is indicated by a colour change from yellow to brick-red.[9]

Quantitative Method (Complexometric Titration)

The quantitative determination employed a modified Deniges complexometric titration method. The distillate was treated with 8 mL of NH_4OH (to retard the precipitation of silver iodide) and 5 mL of 5% KI indicator. The solution was titrated with standard $AgNO_3$ until a permanent turbidity (opalescence) was observed, marking the endpoint. The principle relies on the formation of the stable soluble complex $[Ag(CN)_2]^-$, where turbidity appears once all cyanide has reacted and excess Ag^+ precipitates as AgI.[10]

RESULTS AND DISCUSSION

Qualitative Analysis

Qualitative cyanide analysis data obtained from *gethuk* samples with 5 variants of KSCN concentration additions can be seen in Table-1. This is indicated by a colour change in the picrate paper from yellow to brick red or brownish red after heating, which shows that the sample contains cyanide. Qualitative analysis was performed by grinding the *gethuk* sample with the aim of extracting the active components in the sample without heating or at room temperature, which is capable of breaking down plant cell walls so that they dissolve in the solvent.[11,12]

Table-1: Qualitative Analysis Data of Cyanide

Sample	KSCN Concentration	Result
Gethuk	0.04%	(-)
	0.03%	(-)
	0.02%	(-)
	0.01%	(-)
	0.002%	(-)

The qualitative cyanide test on the *gethuk* sample showed that there was no change in color to brick red at any of the tested concentrations of KSCN, which were 0.04, 0.03, 0.02, 0.01, and 0.002. The brick red color change usually shows that cyanide ions are present, so if there's no colour change, it means cyanide wasn't found in the *gethuk* sample. The qualitative cyanide test on the gethuk sample showed that there was no change in colour to brick red at any of the tested concentrations of KSCN, which were 0.04, 0.03, 0.02, 0.01, and 0.002. The brick red color change usually shows that cyanide ions are present, so if there's no color change, it means cyanide wasn't found in the gethuk sample.[12] From the results of the experiment done on the gethuk sample, the test showed either no cyanide or a small amount of cyanide, but the levels were very low. A drop in cyanide levels in a sample might happen because of steps like washing, soaking, boiling, steaming, or frying. Cassava, which is the main ingredient in gethuk, has compounds that can create HCN. When you heat or boil something, it can lower the amount of cyanide. This is because heat stops the enzymes that make HCN, and any HCN that's already there can escape into the air or mix into the boiling water.[15] Thus, the HCN level in cassava is lower after processing. The qualitative test results indicated a negative response for cyanide in the *gethuk* sample, suggesting that free cyanide compounds were undetectable within the detection limit of the applied method. This finding aligns with the characteristics of the traditional *gethuk* preparation process using sweet cassava, which typically involves peeling, soaking, boiling in an open pot, and discarding the cooking water. These steps facilitate the hydrolysis of cyanogenic glycosides (particularly linamarin) and the volatilisation of hydrogen cyanide (HCN), thereby reducing the cyanide residue to levels below the detection threshold of the qualitative test.[16] Methodologically, a negative result should be interpreted as 'not detected' within the sensitivity of the test rather than as definitive evidence of complete absence. The reliability of this conclusion is influenced by the appropriateness of sample preparation, reaction time and conditions, and quality control measures. Food matrices with high fat or sugar content, such as *gethuk* topped with grated coconut and sugar, generally do not interfere with the picrate paper test; however, maintaining optimal pH, temperature, and aeration during reaction incubation remains crucial to prevent false negatives. When proper procedures are applied, the negative cyanide result in *gethuk* reinforces the assumption that the use of sweet cassava and adequate cooking processes effectively reduces exposure risk. From a health implications perspective, these results support that *gethuk* is safe for regular consumption, provided that hygienic production practices and proper cooking techniques are maintained, including the selection of

sweet cassava, soaking, boiling in an open pot, and discarding the cooking water. For continuous quality monitoring, periodic qualitative screening is recommended for different production batches, particularly when the raw material source changes or during seasonal harvest variations. If required for regulatory or research purposes, quantitative confirmation may be conducted using validated methods, such as micro-diffusion followed by spectrophotometric analysis, to ensure that residual cyanide levels remain well below established safety limits.[17,18]

Quantitative Analysis

Quantitative analysis data obtained from *gethuk* samples with 5 variants of KSCN addition concentrations can be seen in Table-2, with initial treatment weighing 20 grams of samples followed by maceration for 2 hours. The distillate obtained was placed in an Erlenmeyer flask filled with 20 ml of 2.5% NaOH solution. Eight millilitres of ammonia solution was added to the distillate because the titration endpoint occurred too quickly. Therefore, before titration, ammonia needed to be added to form the soluble substance Ag(NH3)2+, which would slow down the precipitation of silver iodide (KI). Then, 5 ml of KI solution was added to serve as an indicator. Next, titration was carried out using an $AgNO_3$ solution that had been standardised with NaCl in order to determine the actual normality of the $AgNO_3$ solution.[19,20] The method used in the cyanide content determination process is modified Deniges complexometric titration because cyanide ions react with silver nitrate solution to form stable AgCN complexes, as indicated by the occurrence of turbidity.[21,22]

Table-2: Quantitative Cyanide Analysis Data

Sample	KSCN Concentration	Replication	Titration volume (ml)	Cyanide levels (mg)	Average level (mg)
Gethuk	0.1%	1	0.9	0.0064	0.007
		2	1.1	0.0076	
		3	0.8	0.0057	
	0.075%	1	0.6	0.0042	0.004
		2	0.6	0.0042	
		3	0.4	0.0028	
	0.05%	1	0.6	0.0042	0.004
		2	0.4	0.0028	
		3	0.6	0.0042	
	0.025%	1	0.5	0.0035	0.003
		2	0.5	0.0035	
		3	0.4	0.0028	
	0.005%	1	0.3	0.0021	0.003
		2	0.4	0.0028	
		3	0.5	0.0035	

From the table showing the cyanide levels in traditional *gethuk* food samples, it can be seen that there was no significant increase in cyanide levels. As shown in Table-2, the titration volumes recorded for the gethuk samples were extremely low, corresponding to calculated values well below the established Limit of Detection (LoD) of 14.43 mg. Therefore, the exact cyanide levels could not be reliably quantified and are reported as not detected (< LoD). As the KSCN concentration increases, there is no significant difference in the concentration variation. Complexometric titration demonstrates limited sensitivity for trace analysis in processed food samples like gethuk, with relatively high Limit of Detection (LoD) values.[11,23] Processes such as washing, boiling, or steaming are factors that can reduce cyanide content. During the washing process, water causes the linamarin compound to undergo hydrolysis and produce cyanide acid that is soluble in water. When the wash water is replaced, the HCN dissolved in the water is also discarded. During the boiling or steaming process, the enzymes linamarase and glucosidase become inactive, thereby halting the formation of cyanide acid. Therefore, cyanide is not produced.[19,24] Globally, organisations such as the Codex Alimentarius Commission (CAC), supported by the FAO and WHO, as well as regulations within the European Union (EU), have established maximum limits for cyanide content (expressed as hydrogen cyanide/HCN) in certain food products. Because cassava—the primary ingredient of *gethuk*—naturally contains cyanide, this commodity is subject to specific standards. The Codex

Alimentarius Commission sets a maximum limit of 10 mg/kg for cassava flour and 2 mg/kg for gari (another processed cassava food product). Meanwhile, European Union regulations (Regulation (EU) 2022/1364) specify more detailed thresholds: 50 mg/kg for fresh, peeled cassava root and 10 mg/kg for cassava flour and tapioca flour.[25–27] The quantitative analysis results showed that the cyanide content in the *gethuk* sample was below the detection limit (LoD) of the complexometric titration method (< 14.43 mg). Therefore, the specific concentration could not be quantified using this method. Although the exact cyanide level was not detectable, the product's safety is supported by the traditional processing steps applied in *gethuk* preparation—namely, peeling, washing, and steaming. These processes have been proven effective in reducing cyanogenic compounds to very low levels, thereby ensuring food safety compliance when compared with the maximum permissible limit for cassava-derived products (10 mg/kg) as established by the Indonesian National Standard (SNI) and the European Union.[28] Consequently, determining the detection limit of cyanide compounds in the traditional Indonesian food *gethuk* directly advances Sustainable Development Goal-3 (SDG-3) by safeguarding consumers against potential dietary toxicity. By validating that the residual cyanogen levels are maintained well below hazardous thresholds, this analytical evaluation actively promotes good health and well-being through evidence-based food safety monitoring.[29,30]

Determination of Detection Limits in Traditional *Gethuk* Food

The cyanide levels obtained from samples of the traditional *gethuk* food production process, to which KSCN had been added at varying concentrations of 0.1%, 0.075%, 0.05%, 0.025%, and 0.005%, were then used to create a standard curve showing the relationship between KSCN concentration and cyanide levels in the samples.[31]

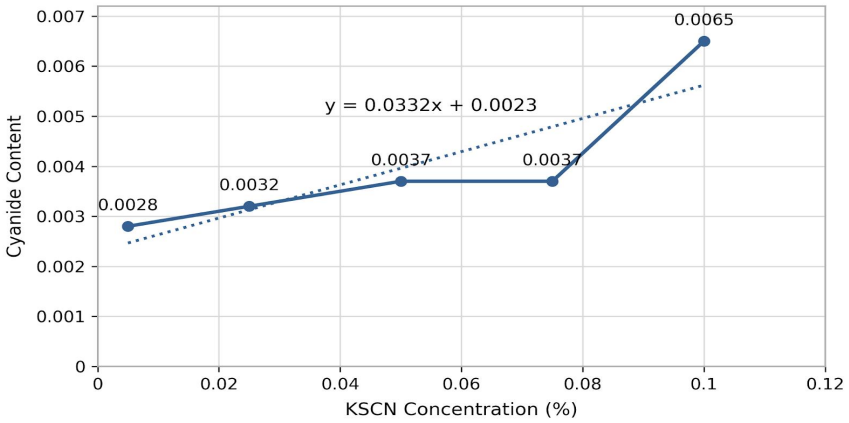

Fig.-1: Calibration Curve

From the curve above, the regression equation for the traditional food sample *gethuk* was obtained as y=0.0332x+0.0023 with a correlation coefficient (R^2) of 0.75. In the linear regression equation derived from the traditional food sample *gethuk* with variations in KSCN additions of 0.1%, 0.075%, 0.05%, 0.025%, 0.005% , the LoD (limit of detection) value was determined to be 14 mg. These results indicate that the minimum cyanide concentration in traditional *gethuk* food with KSCN addition detectable by the complexometric titration method is at a concentration of 14 mg. The results demonstrated that the detection limit for cyanide in the traditional food sample *gethuk,* was relatively high. This is due to the fact that *gethuk* undergoes processing steps such as washing and boiling, which significantly reduce cyanide content. Therefore, the complexometric titration method used in this study could only detect cyanide at relatively high concentration levels. Complexometric titration methods are generally considered less sensitive for trace-level analysis compared to spectrophotometric techniques. This limitation arises from their dependence on visual or colourimetric endpoint detection, which inherently restricts the accuracy and precision of measurements at very low analyte concentrations. In contrast, spectrophotometric methods offer superior sensitivity and lower detection limits due to their ability to measure subtle variations in absorbance associated with trace amounts of metal ions.[23,32]

CONCLUSION

Cyanide compounds were not detected in the traditional Indonesian food *gethuk* through either qualitative picrate paper testing or quantitative complexometric titration. The cyanide content in all tested samples remained below the method's limit of detection (LoD) of 14.43 mg, which is equivalent to 700 mg/kg. While these findings suggest that the traditional processing of *gethuk* effectively minimises acute cyanide exposure, the relatively high LoD indicates that complexometric titration is insufficiently sensitive to verify strict compliance with the Indonesian National Standard (SNI) limit of 1 mg/kg. In alignment with Sustainable Development Goal-3 (SDG-3) to ensure good health and well-being, this study emphasises the necessity of implementing more sensitive analytical techniques, such as spectrophotometry, for future food safety monitoring. Enhancing the precision of detection limits is a vital step in safeguarding public health and ensuring the long-term safety of traditional food staples against chronic toxicological risks.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3: Good Health and Well-Being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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