Application Note
No. 241/2018

Hydrogen cyanide determination in food and feed

KjelFlex K-360:
Total glycosidic hydrocyanic acid in food and feed.

1. Introduction

Cyanogenic glycosides occur in many food and feed products like almonds, bamboo shoots, cassava, lima beans, sorghum and stone fruits and are formed of cyanohydrins which have been stabilized by glycosylation [1].

In the glycosylated form, the cyanogenic glycosides are supposed to be nontoxic, but when digested, the intestinal flora enzymes decompose the cyanogenic glycosides into hydrogen cyanide (HCN) [2] which is highly toxic to humans. The LD50 for ingestion of HCN is only 50° – 200 mg, or 1 – 3 mg HCN / kg of body weight [3].

A cyanogenic glycoside containing food of high economic importance is cassava, also known by the names manioc, yucca and tapioca. The predominant cyanogenic glycoside in cassava is linamarin. Also well-known are the seeds of apricots, bitter almonds and peaches which contain amygdalin as the major cyanogenic glycoside [1].

Cyanogenic glycoside levels can vary with climatic conditions, cultivar, processing level and plant part. Typical HCN levels for some plant materials and their major cyanogenic glycoside composition are shown in Table 1.

Here, we describe a method for the quantitative determination of hydrogen cyanide after enzymatic hydrolysis of the cyanogenic glycosides in almonds, bakery products, kernels, persipan (apricot kernel paste) and marzipan (almond paste) raw mixtures using amygdalin as a model compound [4]. The method is in accordance with the international Standard ISO 2164-1975 and the AOAC Method 915.03 [5 – 6].

Table 1: Major cyanogenic glycosides in selected plant materials and their HCN levels [7 – 9].

Food	Major cyanogenic glycoside	Hydrogen cyanide content [mg HCN / kg]
Bitter almond	Amygdalin	4700
Apricot (kernel)	Amygdalin	89 – 2170 (2.2 in juice)
Apple (seeds)	Amygdalin	690 – 790
Plum (kernel)	Amygdalin	696 – 764
Peach (kernel)	Amygdalin	710 – 720
Nectarine (kernel)	Amygdalin	196 – 209
Cherry (juice)	Amygdalin	4.6 in juice
Cassava (root)	Linamarin	15 – 1000
Flax (seed meal)	Linamarin, linustatin, neolinustatin	360 – 390
Bamboo (young shoots)	Taxiphyllin	100 – 8000
Sorghum (leaves)	Dhurrin	750 – 790
Giant taro (leaves)	Triglochinin	29 – 32

2. Equipment

Equipment:

- BUCHI K-360 KjelFlex, equipped with acid resistant pump
- BUCHI Mixer B-400
- BUCHI Sample tubes 500 mL
- Metrohm Titrino plus 877
- BUCHI Cyanide caps, (order number 11067871, see Figure 1)
- Volumetric pipettes
- Analytical balance (accuracy ± 0.1 mg)
- Magnetic stirrer
- Incubator oven adjustable to 38 °C

Figure 1: BUCHI Cyanide cap.

For titration:

- Volumetric flask 500 mL
- Volumetric flask 200 mL
- Glass cylinder 500 mL
- Glass beaker 600 mL
- Funnel
- Filter 595 ½ Ø 150 mm (Schleicher & Schuell, 10311645)

3. Chemicals and materials

Chemicals:

- Anti-foam agent, e.g. stearic acid, Fluka (85680)
- Sodium acetate solution (20 g/L); pH adjusted to pH 5.0 ± 0.5 with acetic acid
- Sodium acetate working solution; dilute the sodium acetate solution with water 1:9

For titration:

- Nitric acid, (P_{20} 1.38 g/mL ≈ 65 %)
- Silver nitrate, 0.02 N standard volumetric solution, Fluka (34296)
- Ammonium thiocyanate, 0.1 N standard volumetric solution, Fluka (35036), diluted with deionized H_2O to a concentration of 0.02 N (1:5)
- Saturated ammonium iron (III) sulphate solution, Merck (3776)
- Indicator; mix one part of the nitric acid and one part of saturated ammonium iron (III) sulphate solution by volume (1:1)

For a safe handling please pay attention to all corresponding MSDS.

4. Samples

For the determination of cyanogenic glycosides, amygdalin (purity 99.5 %) was used as a reference substance. Amygdalin is the major cyanogenic glycoside in many seeds of the rosaceae species, e.g. apricots, bitter almonds and peaches. Sweet almonds are negligible low in amygdalin.

- Amygdalin 99.5 %, SIGMA (A6005)

Whole, sweet almonds (obtained by local super market)

Sweet almonds are necessary for the enzymatic hydrolysis as an enzymatic catalyst.

5. Procedure

The procedure to determine the HCN content of a sample includes the following steps:

- Enzymatic hydrolysis of the sample which converts cyanogenic glycosides into HCN
- Distillation of the sample
- Manual titration of the distillate (direct titration or back titration) to color change with the help of the titrator Metrohm Titrino plus 877

NOTE: If the enzymatic activity of the sample itself seems insufficient or unknown (e.g. dried, processed samples), enzymatic hydrolysis should be the preferred method. In this application note, the enzymatic hydrolysis was the preferable method.

NOTE: The direct titration needs less volumetric solutions and is faster. However, to find the color detection point of the direct titration requires more practice (from clear → opaque) than applying back titration (from clear → orange). In this Application Note, back titration was the preferable titration method.

Due to the toxicity of HCN and its low boiling point (38 °C), the use of sealing BUCHI Cyanide caps is strongly recommended. In addition, no analyte loss will occur during enzymatic hydrolysis. The Cyanide caps were tested to resist an overpressure of 1 bar.

5.1 Enzymatic hydrolysis

The aim of the enzymatic hydrolysis is to convert the cyanogenic glycosides into HCN. A substrate is added to the sample which contains the specific hydrolysis enzymes to break down the glycosidic binding between the sugar molecule and the cyanide of the sample. Therefore, a suitable substrate must be evaluated depending on the predominant cyanogenic glycoside in the sample, e.g. linamarin or amygdalin.

If amygdalin or linamarin is the main cyanogenic glycoside, sweet almonds or fresh milled cassava root can be used, respectively, as they contain active hydrolysis enzymes.

- Weigh in approximately 2.00 ± 0.01 g of grinded sweet almond in a 500 mL BUCHI Sample tube
- Weigh in the amygdalin in the 500 mL sample tube (see Table 2)
- Add 50 mL of water
- Insert the BUCHI Cyanide cap into the sample tube until stop
- Turn the knob hand-tight. In doing so, the black seal ring is pressed to the glass for hermetical tightness (Figure 2)
- Check if the knob cannot be lifted anymore
- Leave it for 2 h in the incubator at 38 °C
- After incubation, cool the tube and its content in an ice-water bath.

It is important to cool-down the tube to room temperature before the distillation to make sure that the released HCN is not gaseous anymore. The Cyanide caps are removed just before distillation.

Table 2: Weight of sample standard (amygdalin) and catalyst (sweet almond).

Sample	Sweet almond [g]	Amygdalin [g]
Blank	2.0016	-
Amygdalin sample 1	2.0083	0.0134
Amygdalin sample 2	2.0058	0.0140

Figure 2: Sample tube 500 mL with BUCHI Cyanide cap for hermetical closing.

5.2 Distillation

After the enzymatic hydrolysis:

- Remove the Cyanide caps just before distillation
- Add a spatula of anti-foam agent (stearic acid) to the sample tube
- Connect the sample tube immediately to the distillation unit KjelFlex K-360
- Dose 90 mL of sodium acetate working solution using the “reagent pump” directly to the sample tube. The method used for distillation is listed in Table 3
- Distil the sample for 12 minutes (720 s) with 100 % steam power
- Collect the distillate in a 500 mL volumetric flask containing 20 – 50 mL of the 0.02 N silver nitrate solution, containing 1 mL of the nitric acid (1.38 g/mL)

The volume of silver nitrate solution used depends on the presumed content of hydrogen cyanide. For low and high cyanide content samples use 20 mL and up to 50 mL, respectively. The silver nitrate solution must be dosed exactly with a volumetric pipette. If the content is unknown, start with 20 mL silver nitrate solution. If the titration volume is ≤ 1 mL increase the volume of silver nitrate solution about + 10 mL.

NOTE: The addition of nitric acid to the silver nitrate volumetric solution avoids possible errors due to the presence of carbonate and phosphate ions which, under basic conditions, would react with the silver ions.

Distilled HCN reacts with the silver nitrate to silver cyanide forming a white precipitate (Reaction 1).

$$HCN\ (\text{from distillation}) + AgNO_3 + HNO_3 \rightarrow AgCN + 2HNO_3 \qquad (1)$$

Table 3: Parameters for distillation and titration with the KjelFlex K-360.

H_2O volume	0 mL	Distillation time	720 s
NaOH volume	0 mL	Titration type	No titration (manual)
H_3BO_3 volume	0 mL	Stirrer speed distillation	0
Reagent (sodium acetate working solution) volume	90 mL	Stirrer speed titration	0
Reaction time	5 s	Aspiration sample tube	No
Steam output	100 %	Aspiration receiving vessel	No

5.3 Back titration

- Filter the captured distillate (≈ 450 mL) through a dry filter, collect the filtrate in a dry 500 mL volumetric flask
- Rinse the flask with d H_2O, transfer it on the filter
- Wash the filter with d H_2O, until it reaches ≈ 495 mL
- Dilute the filtrate to the 500 mL mark with distilled water and mix thoroughly

NOTE: The filtration after distillation is needed, due to lower solubility of silver thiocyanate compared to silver cyanide. During titration, thiocyanate can replace cyanides in the existing precipitate. To avoid this reaction, the precipitated silver cyanide is removed by filtration before titration.

- Measure 200 mL of the filtrate (sample) in a 200 mL volumetric flask
- Transfer the 200 mL sample into a glass beaker
- Add 2 mL of the indicator (1 : 1 nitric acid 1.38 g/mL with saturated ammonium iron (III) sulfate solution) to the sample
- Titrate manually with the 0.02 N ammonium thiocyanate standard volumetric solution until a permanent orange coloration appears. The color changes during titration are shown in Figures 3 – 5

As soon as there is no more silver nitrate, the titrant reacts with the indicator and an orange coloration appears and indicates the endpoint of titration (see Reactions 2 and 3).

$AgNO_3$ (excess) + NH_4SCN (titrant) → AgSCN + NO_3^- + NH_4^+ (2)

$3NH_4SCN$ (titrant) + $NH_4Fe(SO_4)_2$ (indicator) → $Fe(SCN)_3$ (orange) + $4NH_4^+$ + $2SO_4^{2-}$ (3)

- Repeat the titration with another 200 mL and take the average of the two titrations

Figure 3: Distillate before titration with 0.02 N ammonium thiocyanate standard volumetric solution (transparent).

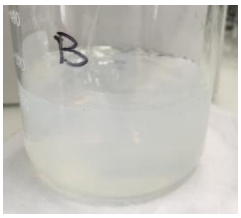

Figure 4: Distillate during titration with 0.02 N ammonium thiocyanate standard volumetric solution (opaque).

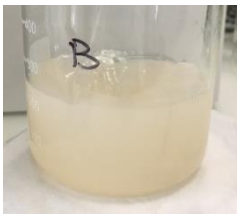

Figure 5: Distillate after titration with 0.02 N ammonium thiocyanate standard volumetric solution (orange).

5.4 Calculations

The content of cyanogenic glycosides, expressed in milligrams of hydrogen cyanide (HCN) per kilogram sample is equal to [5]:

$$27.03\frac{\text{g}}{\text{Mol}} * 0.02\frac{\text{Mol}}{\text{L}} * 1000 * \frac{(\text{V0}-\text{V1})\text{mL}}{1000} . \frac{500\text{ mL}}{200\text{ mL}} / \frac{\text{Sample [g]}}{1000} = \frac{\mathbf{1350\ (V0-V1)}}{\boldsymbol{m}} \quad (1)$$

Molecular weight HCN: 27.03 g/Mol
Titrant concentration 0.02 Mol/L

m: mass [g] of the test sample
V0: volume [mL] of the ammonium thiocyanate solution (0.02 N) used for the blank
V1: volume [mL] of the ammonium thiocyanate solution (0.02 N) used for the sample determination

If the standard volumetric solution used are not exactly the concentrations indicated in the list of chemicals, a suitable correction factor should be used in calculating the results.

If less than 10 mg of hydrocyanic acid per kilogram of sample (< 10 ppm) are measured, consider the sample practically free from cyanogenic glycosides.

6. Results and discussion

The results of the HCN determination in amygdalin are presented in Table 4.

Table 4: Results of the HCN determination in amygdalin.

	Sweet, milled almonds [g]	Amygdalin [g]	Titrant volume 1 [mL]	Titrant volume 2 [mL]	Average [mL]	Calculation [mg HCN /kg]	Calculation [g HCN /kg]	Recovery [%]
Blank	2.0016	-	8.1450	8.1550	8.1500	-	-	-
Amygdalin 1	2.0083	0.0134	7.5920	7.5666	7.5793	57495.9	57.50	97.5
Amygdalin 2	2.0058	0.014	7.5440	7.5240	7.5340	59400.0	59.40	100.7

The theoretical HCN content of amygdalin is 5.91 % or 59.10 g HCN/kg. The recovery rate of the two measured amygdalin samples was 99.1 % (n=2).

7. Comparison to standard methods

Table 5 shows the differences between the applied method and the international Standard ISO 2164-1975 [5] and AOAC 915.03 [6].

Table 5: Comparison to standard methods ISO 2164-1975 and AOAC 915.03.

	ISO 2164-1975 AOAC Method 915.03	BUCHI method	Influence
Sample amount analyzed	20 g	0.5 g – 20 g depending on expected HCN content (see Table 1).	For additional safety reasons, less sample should be used if cyanide concentration is unknown.
Distillate volume collected [mL]	Approx. 100 mL	Approx. 450 mL	To make sure all cyanide was distilled.
Filtration	After the distillation the 500 mL volumetric flask is filled up to the mark. Afterwards it's filtrated.	After distillation the collected ≈ 450 mL were filtrated. The 500 mL volumetric flask, where the distillate was captured, was rinsed three times with d H_2O. Further, the filter was washed with d H_2O. After filtration, the filtrate was filled up to the mark of the 500 mL volumetric flask with d H_2O.	Recovery rate increased because of the washing step with d H_2O of the receiving volumetric flask and the filter.
Volume used for titration [mL]	2 x 250 mL	2 x 200 mL	To ensure that both determinations contain sufficient and the same volume, 2 x 200 mL was used of the 500 mL distillate.
Equation for cyanogen content calculation	(1080 (V0-V1))/m	(1350 (V0-V1))/m	Because of the reduced volume for titration used (2 x 200 mL instead of 2 x 250 mL), the equation was adapted.

8. Conclusion

Due to the toxicity of hydrogen cyanide (HCN), it is important to have efficient methods to evaluate the cyanogenic potential of food and feed products.

With the optimized method presented in this study and the usage of the BUCHI Cyanide caps, a recovery rate for HCN of 99.1 % in amygdalin was achieved. Additionally, the BUCHI Cyanide caps sealed the sample tubes hermetically which allowed to work under save conditions.

9. References

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