Quality Assurance and Safety of Crops & Foods, 2015; 7 (4): 501-507

Wageningen Academic Publishers

Single laboratory method validation for cyanide in beans with insufficient levels of β-glucosidase activity

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Received: 1 August 2013 / Accepted: 27 May 2014
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RESEARCH ARTICLE

Abstract

Methods to determine cyanide in beans, such as the AOAC OMA 915.03 utilise endogenous enzyme linamarase (β-glucosidase) to liberate hydrogen cyanide (HCN). However, several beans are found to contain insufficient levels of β-glucosidase activity to evaluate their levels of cyanide. This implies some beans indicate false negative using official methods. Therefore, we developed a method to analyse cyanide in beans which lack linamarase activity, and a single laboratory validation was performed. This method used 3 U of linamarase and samples were maintained at 38 °C for 24 h to hydrolyse the cyanogenic glucoside in the beans. After hydrolysis, water and antifoam were added, then, this mixture was distilled to trap cyanide in an alkaline solution. The trapped cyanide was then quantified using a 4-pyridinecarboxylic acid-pyrazolone colouring method. To validate this method, non-cyanide containing blank bean (cowpea) was spiked with potassium cyanide (KCN) from 1.0 to 500 mg/kg and linamarin from 5.0 to 25 mg/kg HCN equivalent and analysed. The recovery of cyanide from KCN ranged from 82 to 91%, with relative standard deviation (RSD) of 1.2 to 3.7%. The recovery of cyanide from linamarin ranged from 82 to 83%, with RSD of 6.2 to 8.0%. Lima bean, with naturally containing cyanogenic glucosides, was mixed with non-cyanide containing cowpea to cyanide levels of 5.0 to 40 mg/kg. Standard deviations of 'within day' and 'between days' recoveries using these samples were 2.8 to 4.6% and 3.0 to 7.8% at 5.0 to 40 mg/kg HCN level, respectively. The release of HCN from six beans of *Phaseolus lunatus* was measured either with and without addition of enzyme. Five of the beans had significant differences of detected HCN with versus without enzyme addition.

Keywords: cyanogenic glycoside, lima bean, linamarase, 4-pyridinecarboxylic acid-pyrazolone method

1. Introduction

More than 70 different cyanogenic glycosides (Poulton, 1990) are contained in more than 2,500 plants (Bak *et al.*, 2006). For example, amygdalin contained in the seed of bitter almonds and apricots and linamarin in cassava are well known. Cyanogenic glycosides release toxic hydrogen cyanide (HCN) in animals when ingested. The levels of cyanogenic glycosides in plants can be reduced by soaking in water (Kawamura *et al.*, 1993), sun-drying (Mlingi and Bainbridge, 1994; Nambisan and Sundaresan, 1985), boiling (Kawamura *et al.*, 1993) and heap-fermentation (Cardoso *et al.*, 1998; Essers *et al.*, 1995). Therefore plants that contain cyanogenic glycosides can be used as foods after processing. However, when plants containing cyanogenic glycosides are consumed without successful detoxification, the animal may suffer abdominal pains, vomiting, dizziness, nausea, palpitation, weakness, diarrhoea, headache, difficulty in vision and occasionally death (Akintonwa *et al.*, 1994; Mlingi *et al.*, 1992). For example, konzo disease which is suspected to be caused by cyanide released from ingested bitter cassava has been reported from several countries in Africa (Banea *et al.*, 2012). In 1948, cyanide intoxication for human occurred in Japan by sultani beans that were imported from Burma (Japan Food Hygiene Association, 2013). Because of these incidences of cyanide poisoning, the Codex Alimentarius Commission set a limit of 10 mg/kg HCN for edible cassava flour (Codex Alimentarius, 1989). Most countries follow the Codex advice but some countries such as Indonesia have set their own limits of 40 mg/kg (Damardjati *et al.*, 1993). The Codex sets limit of cyanide only in cassava flour, however, in Japan, cyanide in

raw beans and bean paste is controlled under detection limit (Japan Food Hygiene Association, 2005). Also, in Japan, beans containing less than 500 mg/kg HCN were allowed for processing bean paste because the bean paste process could remove cyanide by soaking and refining in water.

Methods to determine cyanide in beans, such as the AOAC Official Method of Analysis (OMA) 915.03 (AOAC International, 2005) and the Japanese Official Method (Japan Food Hygiene Association, 2005), utilise the endogenous enzyme in beans (linamarase; β-glucosidase EC 3.2.1.21) to liberate HCN from its conjugates. However, several beans with high levels of cyanide compounds contain insufficient levels of β-glucosidase for this purpose. Thus, in these beans, the cyanide level may not be able to be accurately quantified by these methods. Therefore, we developed a method to analyse cyanide in beans that lack sufficient linamarase activity, based on the pyridinecarboxylic acid-pyrazolone method (Japan Food Hygiene Association, 2005) and then validated the method.

2. Materials and methods

Chemicals

Antifoam SI, linamarin, linamarase (from butter bean) and p-nitrophenyl-β-D-glucopyranoside were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). Neutralit pH indicator strips for pH 0 to 14 was purchased from Merck (Darmstadt, Germany). Other chemicals were purchased from Kanto Chemical Co. Ltd. (Tokyo, Japan). For potassium cyanide (KCN) stock solution, KCN was dissolved in 0.27% KOH aqueous solution, and this solution stored at 4 °C and used within 1 week after preparation. KCN stock solution was diluted with 0.27% KOH solution to prepare HCN concentration at 0.01 to 1 μg/ml.

Pyridinecarboxylic acid-pyrazolone solution was prepared from two solutions. One solution was prepared by dissolving 1.5 g of 4-pyridinecarboxylic acid in 20 ml of 1 mol/l NaOH aqueous solution and the pH was adjusted to 7.0 by 1 mol/l HCl. Another solution was prepared by dissolving 0.3 g of 1-phenyl-3-methyl-5-pyrazolone in 20 ml N,N-dimethylformamide. These two solutions were mixed then diluting by distilled water to 100 ml. This solution was stored at 4 °C in the dark and used within 2 week. p-Nitrophenyl-β-D-glucopyranoside was dissolved in 0.05 mol/l pH 5.2 citrate buffer and adjusted to 2 mmol/l. Linamarase solution was prepared by dissolving in pH 5.9 citrate buffer and adjusted to 3 U/ml. Citrate buffer was prepared by dissolving 128.1 g of citric acid and 64.4 g of NaOH in 1 l of distilled water, and diluting 10 fold by distilled water and its pH was adjusted to 5.9 when used.

Samples

Six lima bean (*Phaseolus lunatus*) samples were purchased at markets in California in the USA, Nagano and Niigata in Japan. Lima bean and cowpea (*Vigna unguiculata*) used in method validation were grown in the experimental field in Shinshu University, Nagano, Japan. Yellow and black soybean (*Glycine max*), adzuki bean (*Vigna angularis*) and tebo bean (*Phaseolus vulgaris*) produced in Hokkaido, Japan and tiger bean (*Phaseolus vulgaris*), pea (*Pisum sativum*), broad bean (*Vicia faba*) and runner bean (*Phaseolus coccineus*) produced in Nagano, Japan were purchased at local markets. 50 g of beans was ground by centrifuge mill with 1 mm mesh (P-14; Fritsch, Idar-Oberstein, Germany) and stored at -20 °C until used.

Measurement of β-glucosidase activity in beans

The measurement of β-glucosidase activity in beans was based on the method by Uda *et al.* (1984). In brief, 5 g of sample was added 50 ml of distilled water, crushed and extracted for 3 min by 16 speed blender (Oster, Boca Raton, FL, USA). The extract was centrifuged for 40 min at 22,000×*g*. Ammonium sulphate fraction (45 to 85% saturation) was dissolved to 0.05 mol/l pH 5.2 citrate buffer. The solution was dialysed overnight and changed outside solution, 0.05 mol/l pH 5.2 citrate buffer, for 4 times. The precipitation was removed by centrifugation for 15 min at 10,000×*g*, and the supernatant was used as enzyme solution.

To measure β-glucosidase activity, 20 μl of enzyme solution was added to 0.5 ml of 2 mmol/l p-nitrophenyl-β-D-glucopyranoside dissolved in the same citrate buffer in a small test tube. The test tube was then sealed tightly with a silicone stopper and incubated for 10 min at 38 °C. The reaction was stopped by adding 3.5 ml of 0.2 mol/l pH 9.8 borate buffer. The absorbance of the solution was measured at 400 nm by spectrophotometer (UV-1600PC; Shimadzu, Kyoto, Japan). β-Glucosidase activity was calculated as follows:

$$\beta\text{-glucosidase activity (U/dw(g))} = (A_{test} - A_{control})/10/17.7 \times 4.02/W$$

Where A_{test} = the absorbance with the enzyme solution; $A_{control}$ = the absorbance of control; 10 = the enzyme reaction time (min); 17.7 = milli-molar extinction coefficient of p-nitrophenol; 4.02 = total volume (ml); W = dry weight of the bean in 20 μl of the enzyme solution (g); and dw(g) = dry weight of bean sample.

Release of hydrogen cyanide from beans and distillation

7.5 g of ground bean sample was placed in a 300 ml flat bottom flask. 100 ml of pH 5.9 citrate buffer, 1 ml of 3 U/ml linamarase solution and a few drops of toluene were added

to the flask (Frehner *et al.*, 1990). The flask was then sealed tightly with a silicone stopper, and subjected to continuous stirring at 38 °C for 24 h. After hydrolysis, 50 ml of distilled water and 15 drops of antifoam were added to the flask and mixed. Then the flask was connected to a cooling condenser and distilled directly at about 1.4 ml/min. The distillate was trapped in the 10 ml of KOH aqueous solution. Distillation was stopped when ca. 70 ml of the distillate was collected, and the solution was filled up to 75 ml with distilled water. We compared the pHs of the distillate with and without a bean sample when 0.75, 2 and 3% of KOH were used as trap solutions. Also, the pHs of the mixture of the distillate and phosphate buffer were compared using same trap solutions.

Quantification for cyanide

2 ml of distillate which was diluted two-fold with 0.27% KOH, 1 ml of 0.4 mol/l phosphate buffer and 0.2 ml of 0.05 mol/l chloramine T aqueous solution were mixed in a test tube. After 5 min at room temperature, 1 ml of pyridinecarboxylic acid-pyrazolone solution was added and then the test tube was sealed tightly with a silicon stopper and placed in water bath at 38 °C for 40 min. The absorbance of this solution was measured at 638 nm by spectrophotometer. Concentration of CN was calculated as follows:

$$HCN\ (mg/kg) = \{(A_{test}-A_{control})-b\}/a \times 75/2/1000/0.0075$$

Where A_{test} = absorbance of sample; $A_{control}$ = absorbance of control; b = intercept of calibration curve; a = slope of calibration curve; 75 = volume of distillate (ml); 2 = volume of distillate used for colouring reaction (ml); 1000 = conversion factor from μg to mg; and 0.0075 = amount of bean used for analysis (kg).

When the concentration of cyanide exceeded from the range of the standard curve, the distillate was re-diluted with the 0.27% KOH solution and remeasured. When a white turbidity occurred in the solution, the solution was filtered through a 0.22 μm PTFE syringe filter (OD 13 mm; Labo Labo Company, Tokyo, Japan) and then the absorbance was measured. The standard curve was linear (r^2=1.0) in the range of 0.02 to 2 μg of HCN, which was equivalent to 0.2 to 20 mg/kg of HCN in bean.

Study of enzyme reaction conditions

The amounts of released HCN using 1, 3, 5 and 10 U of linamarase for 4 h of reaction time were determined using a lime bean of low β-glucosidase activity. Also, released HCN amounts after reaction times of 4, 8, 16, 20, 24 and 28 h with 3 U of the enzyme were measured.

Single laboratory validation

This method comprises three steps: (1) an enzyme reaction step to release HCN from cyanogenic glucosides; (2) a distillation step to collect liberated HCN; and (3) an analytical step to determine the cyanide level in a distillate. To estimate the recovery of HCN at the distillation step, a blank bean (cowpea) was spiked with KCN in 0.27% KOH solution at 1.0, 5.0, 10, 100, 500 mg/kg, and subsequently analysed for HCN. To estimate the total recovery of HCN for all steps, cowpea was added with a linamarin in pH 5.9 citrate buffer at 5.0, 10 and 25 mg/kg HCN equivalence and the amount of HCN measured. The relative recovery ratio of HCN was tested at approximately 1.2 to 40 mg/kg cyanide containing sample. These samples were prepared by diluting cyanide-containing lima bean (equivalent to 40 mg/kg HCN) with non-cyanide containing cowpea. Results for these samples were expressed as a relative ratio. Using this mixture, relative standard deviations of HCN 'within day' and 'between days' at 5.0, 10 and 40 mg/kg equivalent HCN levels were determined. Relative standard deviations of 'within day' samples were analysed using 6 replicates in the same day. Relative standard deviations of 'between days' samples were analysed using 6 replicates each day over 3 different days.

3. Results and discussion

Study of the trap solution

First, we used 5 ml of 0.5% KOH aqueous trap solution; however, analysed levels could not be adequately repeated, and the pH of trap solution became low after distillation. On account of this, we studied for an appropriate concentration of KOH solution. Also, 5 ml of trap solution was an insufficient volume to collect HCN, so the trap solution volume was changed to 10 ml.

HCN in water forms a weak acid, so a trap solution at the distillation step should be stable in basic condition. Also, after mixing with phosphate buffer, the pH of the distilled solution should be between 7 to 8 in order to obtain good stable data. When 0.75% KOH solution was used for the trap solution, the pH of distillate without a bean sample was 12. However, the pH of that solution with a bean sample decreased to 11. On the other hand, when 2 and 3% KOH solution was used, there were no differences of pHs of distillates either with and without a bean at each concentration of the trap solutions. When the pH of a mixture of phosphate buffer and the distillate using 0.75 and 2% KOH solution was 7 and 7.5 (well within the range of pH 7 to 8), these samples were acceptable for the next colouring step. However, the mixture of the distillate using 3% KOH solution was pH 9 and outside of the acceptable range. Therefore, we chose 2% KOH solution as a trap solution. Also, detected HCN amounts from the distillate diluted

by two, three and four fold with 0.27% KOH were 10.3, 10.4 and 10.4 mg/kg, respectively. However, the amount of HCN detected from the non-diluted distillate was only ca. 80% of the diluted samples. Therefore, the distillate was diluted at least two folds with 0.27% KOH solution before the colouring step.

Condition of enzyme use for cyanide release

Figure 1 shows the liberated HCN amounts from cyanogenic lima beans containing low β-glucosidase activity which were added with 1, 3, 5 and 10 U of linamarase. The amounts of liberated HCN were 1.5, 2.9, 5.0 and 7.6 μmol, respectively, indicating that the amounts of released HCN were dependent on the amount of added linamarase.

Figure 2 shows the effect of incubation time on the amounts of liberated HCN from beans added with 3 U linamarase. When incubated for 4, 8, 16, 20, 24 and 28 h the amount of HCN released were 6.0, 8.1, 9.7, 9.7, 9.7 and 9.4 μmol, respectively. While the amount of HCN at the originally condition, 10 U and 4 h was 9.5 μmol. This result shows 3 U linamarase with incubation time more than 16 h can liberate an equivalent amount of HCN from bean compared to the original condition of 10 U and 4 h. Therefore we used 3 U linamarase and 24 h incubation time for single laboratory validation because it suited experimental schedule and was more cost efficient.

Single laboratory validation

Single laboratory validation was performed following the scheme in Figure 3. The recoveries of the distillation step were shown in Table 1. The recoveries of HCN from 1.0 to 500 mg/kg KCN spiked blank beans were 82 to 91% and their relative standard deviations (RSDs) were 1.2 to 3.7%. In Table 2, the recoveries of HCN from 5.0 to 25 mg/kg linamarin spiked blank beans were 82 to 83% and their RSDs were 6.2 to 8.0%.

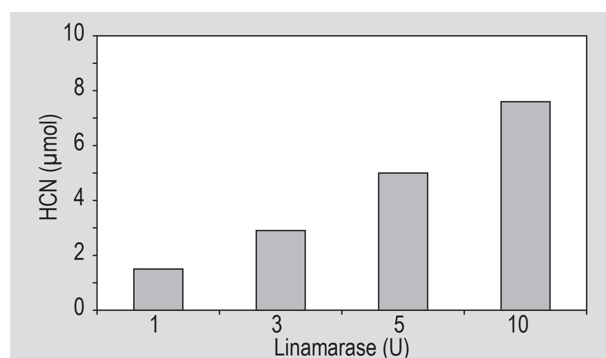

Figure 1. Liberated hydrogen cyanide (HCN) from lima bean in relation to added linamarase. Incubation time of all data was 4 h. A lima bean which β-glucosidase activity of 0.022 U/g (dry weight) was used.

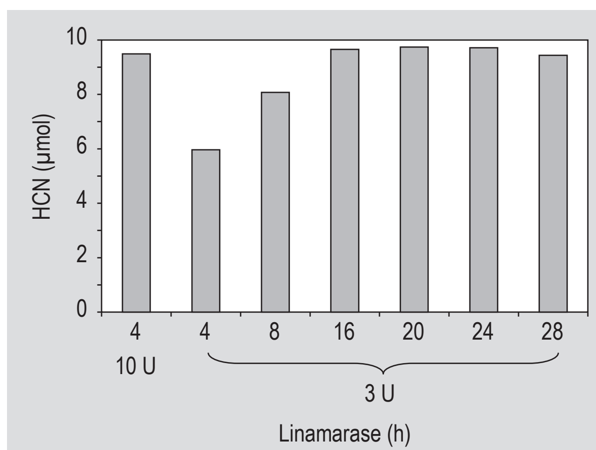

Figure 2. Liberated hydrogen cyanide (HCN) and enzyme reaction period. A lima bean which β-glucosidase activity of 0.046 U/g (dry weight) was used. An incubation time of 4 h and added linamarase of 10 U was compared with an incubation time of 4 to 28 h and added linamarase of 3 U.

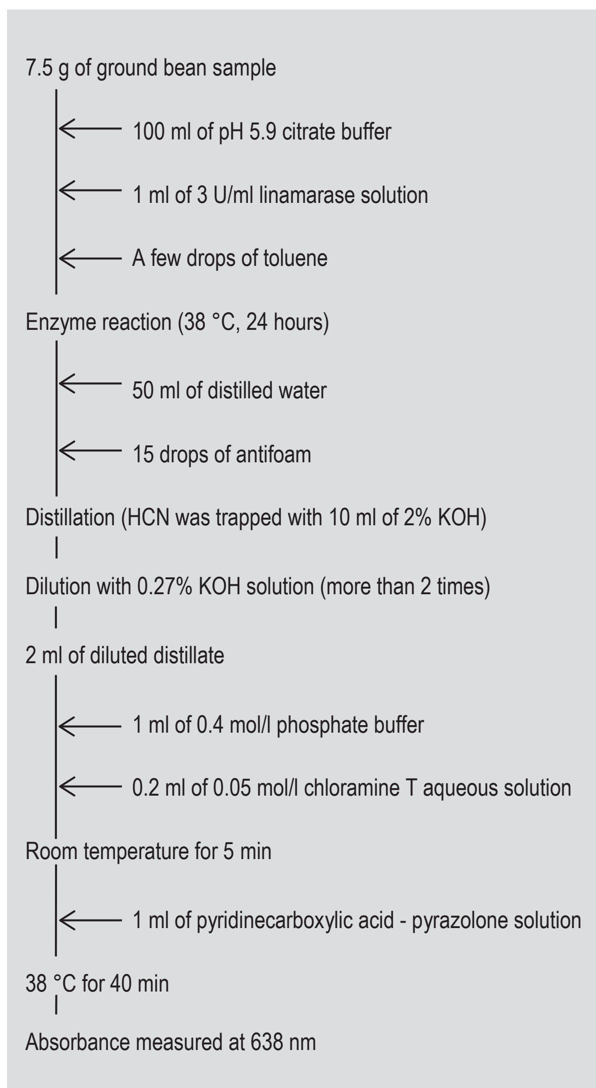

Figure 3. Scheme of cyanide analysis.

Table 1. The recoveries of hydrogen cyanide (HCN) at the distillation step.

	The distillation step	
Spiked HCN (mg/kg)	Recovery (%)[1]	RSD (%)
1.0	83	3.7
5.0	82	2.5
10	86	2.0
100	86	1.2
500	91	2.7

[1] KCN solution was spiked to cowpea at 1.0 to 500 mg/kg HCN equivalent (n=3). RSD = relative standard deviation.

Table 2. The recoveries of hydrogen cyanide (HCN) at the total step.

	The enzyme reaction step	
Spiked HCN (mg/kg)	Recovery (%)[1]	RSD (%)
5.0	82	7.3
10	83	8.0
25	83	6.2

[1] Linamarin solution was spiked to cowpea at 5.0 to 25 mg/kg HCN equivalent (n=3). RSD = relative standard deviation.

Table 3 shows the relative recovery ratio of HCN from cyanide containing lima beans in the range from 1.2 to 40 mg/kg. The recovery ratios were expressed relative to recovery of HCN from 10 mg/kg as 100%. As the results indicate, the recovery ratios were 89 to 106% and no significant difference of recoveries were observed on HCN concentration.

Table 4 shows the relative standard deviations of 'within day' and 'between days' recoveries of HCN for lima bean at 5.0, 10 and 40 mg/kg. Each concentration was analysed 6 times per day for 3 days. Averages of relative standard deviations of within day recoveries were 2.8 to 4.6% and relative standard deviations of between days were 3.0 to 7.8%.

Table 3. The relative recovery ratio of hydrogen cyanide (HCN).

HCN (mg/kg)	Recovery ratio (%)[1]
1.2	101
2.5	106
5.0	101
10	100
20	89
40	98

[1] Recovery ratio is expressed relative to recovery of 10 mg/kg (100%) (n=2).

Analyses of market bean samples

Table 5 shows the results of HCN analysis and its β-glucosidase activity of beans collected from the market. Five out of six samples showed significant differences in HCN levels when the enzyme is added versus not added. These samples also possessed low β-glucosidase activity of 0.024 to 0.25 U/dw(g). A sample with 4.6 U/dw(g) β-glucosidase activity did not show significant differences in the amount of HCN with or without the addition of linamarase. For the samples with low β-glucosidase activity, the ratios of HCN amounts detected with and without linamarase were 19.9 to 30.2%. All these cyanogenic beans showing low β-glucosidase activity were large lima bean.

We also analysed eight bean samples, yellow and black soy bean, adzuki bean, tebo bean, tiger bean, pea, broad bean and runner bean, which were considered containing no cyanogenic glucosides or low cyanogenic glucosides. From these beans, no more than 0.2 mg/kg of HCN was detected even with the addition of linamarase.

Table 4. Relative standard deviation of hydrogen cyanide (HCN) for within day and between days study.

HCN (mg/kg)	Within day (n=6)			Between day (n=3)		
	RSD_r(%)	$PRSD_r$(%)	$HorRat_r$	RSD_r(%)	$PRSD_r$(%)	$HorRat_r$
5.0	4.6 (5.0, 3.7, 5.0)	12	0.38	3.0	12	0.25
10	4.0 (4.4, 2.7, 4.9)	11	0.36	7.8	11	0.71
40	2.8 (2.7, 3.6, 2.2)	9.2	0.30	3.1	9.2	0.34

RSD_r = relative standard deviation for repeatability; $PRSD_r$ = predicted relative standard deviation for repeatability; HorRat = Horwitz ratio.

Table 5. Released hydrogen cyanide (HCN) amount and β-glucosidase activity from *Phaseolus lunatus* with and without addition of the enzyme linamarase.

	Samples		Colour	HCN (mg/kg)		β-glucosidase activity (U/dw(g))
				Linamarase addition		
				Without	With	
USA	California	large lima	white	11.6	48.3	0.058
	California	large lima	white	13.5	79.0	0.056
	California	baby lima	white	65.9	64.8	4.6
Japan	Nagano	large lima	red	6.4	21.2	0.024
	Nagano	large lima	red	6.3	31.6	0.041
	Niigata	large lima	red	9.1	31.9	0.25

dw(g) = dry weight of bean sample.

4. Conclusions

We improved the cyanide analysis method in beans based on the 4-pyridinecarboxylic acid-pyrazolone colouring method. This improved method reduced the use of expensive linamarase from 10 U to 3 U and also used direct distillation instead of steam distillation. A single laboratory validation was performed and the relative standard deviations of within day and between days at 5.0 to 40 mg/kg levels were 2.8 to 4.6% and 3.0 to 7.8%, respectively. We analysed six samples of *Phaseolus lunatus* by this method and five samples showed significant difference between added linamarase versus not added.

Acknowledgements

The authors thank Dr Mark Fukayama for his suggestions during the preparation of this manuscript.

References

Akintonwa, A., Tunwashe, O. and Onifade, A., 1994. Fatal and non-fatal acute poisoning attributed to cassava-based meal. Acta Horticulturae 375: 285-288.

Association of Official Analytical Chemists (AOAC) International, 2005. AOAC official method 915.03 Hydrocyanic acid in beans. In: Horwitz, W. (ed.) Official methods of analysis of AOAC International (18th Ed.). AOAC International, Gaithersburg, MD, USA.

Bak, S., Paquette, S.M., Morant, M., Morant, A.V., Saito, S., Bjarnholt, N., Zagrobelny, M., Jørgensen, K., Osmani, S., Simonsen, H.T., Pérez, R.S., Van Heeswijck, T.B., Jørgensen, B. and Møller, B.L., 2006. Cyanogenic glycosides: a case study for evolution and application of cytochromes P450. Phytochemistry Reviews 5: 309-329.

Banea, J.P., Nahimana, G., Mandombi, C., Bradbury, J.H., Denton, I.C. and Kuwa, N., 2012. Control of konzo in DRC using the wetting method on cassava flour. Food and Chemical Toxicology 50: 1517-1523.

Cardoso, A.P., Ernesto, M., Cliff, J., Egan, S.V. and Bradbury, J.H., 1998. Cyanogenic potential of cassava flour: field trial in Mozambique of a simple kit. International Journal of Food Sciences and Nutrition 49: 93-99.

Codex Alimentarius, 1989, Codex standard for edible cassava flour (Codex Stan 176-1989). Codex Alimentarius, Rome, Italy. Available at: http://www.codexalimentarius.org/input/download/standards/59/CXS_176e.pdf.

Damardjati, D.S., Widowati, S. and Rachim, A., 1993. Cassava flour production and consumers acceptance at village level in Indonesia. Indonesian Agricultural Research and Development Journal 15: 16-25.

Essers, A.J., Ebong, C., Van der Grift, R.M., Nout, M.J.R., Otim-Nape, W. and Rosling, H., 1995. Reducing cassava toxicity by heap fermentation in Uganda. International Journal of Food Sciences and Nutrition 46: 125-136.

Frehner, M., Scalet, M. and Conn, E.E., 1990. Pattern of the cyanide-potential in developing fruits. 1. Implications for plants accumulating cyanogenic monoglucosides (*Phaseolus lunatus*) or cyanogenic diglusosides in their seeds (*Linum usitatissimum*, *Prunus amygdalus*). Plant Physiology 94: 28-34.

Japan Food Hygiene Association, 2005. Standard methods of analysis in food safety regulation, physics and chemistry, 2005. Japan Food Hygiene Association, Tokyo, pp. 707-712.

Japan Food Hygiene Association, 2013. History of Japanese food hygiene 1945-1964. Japan Food Hygiene Association, Tokyo. Available at: http://www.n-shokuei.jp/town/history/nenpyo01.html.

Kawamura, Y., Hikidi, S., Maruyama, K., Uchiyama, S. and Saito, Y., 1993. Fate of cyanogenic compounds in beans during the manufacturing process of bean paste. Journal of the Food Hygienic Society of Japan 34: 80-83.

Mlingi, N.L.V. and Bainbridge, Z., 1994. Reduction of cyanogen levels during sun-drying of cassava in Tanzania. Acta Horticulturae 375: 233-239.

Mlingi, N., Poulter, N.H. and Rosling, H., 1992. An outbreak of acute intoxications from consumption of insufficiently processed cassava in Tanzania. Nutrition Research 12: 677-687.

Nambisan, B. and Sundaresan, S., 1985. Effect of processing on the cyanoglucoside content of cassava. Journal of the Science of Food and Agriculture 36: 1197-1203.

Poulton, J.E., 1990. Cyanogenesis in plants. Plant Physiology 94: 401-405.

Uda, Y., Itoh, T., Kanmuri, M. and Naoi, Y., 1984. Enzymic analysis of cyanogenic glycosides. I. Hydrolysis of cyanogenic glycosides by various β-glucosidases. Eisei Kagaku 30: 290-294.