PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

OPEN ACCESS Pakistan Journal of Nutrition

ISSN 1680-5194
DOI: 10.3923/pjn.2017.914.920

Research Article
Effect of Cellulase Addition on Linamarin Hydrolysis in Cassava (*Manihot esculenta*) Slurry

[1]Maherawati, [2]Muhammad Nur Cahyanto, [2]Yudi Pranoto and [2]Tyas Utami

[1]Faculty of Agriculture, Tanjungpura University, 78124 West Kalimantan, Indonesia
[2]Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Gadjah Mada University, 55281 Yogyakarta, Indonesia

Abstract

Background and Objective: A variable amount of residual cyanide can still be found in processed cassava products, indicating that all of the linamarin in cassava cannot be hydrolysis. Linamarin and its enzyme, linamarase, are located in different cellular locations, therefore, disruption of the cell wall by cellulose could provide contact between linamarin and linamarase. The objective of this study was to improve linamarin hydrolysis by endogenous linamarase in cassava slurry using various concentrations of cellulases. **Methodology:** Cellulases from Celluclast® at concentrations of 0.075 filter paper unit (FPU) mL^{-1}, 0.015 and 0.3 FPU mL^{-1} were added into cassava slurry and then incubated at 50°C for 24 h. During incubation, the reducing sugar, starch and hydrogen cyanide (HCN) contents were analysed and microscopic examination was conducted. **Results:** The reducing sugar content increased at all enzyme loadings, indicating that cellulose hydrolysis occurred. The starch content increased to 15.72 g/100 mL slurry at the highest enzyme loading. Rupture of the cassava cell wall was confirmed by light microscopy and scanning electron microscopy, which showed that the cell wall was damaged and starch granules were freed from the cell. Cell wall degradation allowed linamarin to make contact with endogenous linamarase and produced HCN, which increased at all enzyme loadings. The pattern of increasing HCN content was in accordance with the endogenous linamarase activity. **Conclusion:** The addition of cellulases increased linamarin hydrolysis in cassava slurry up to 79%. This methods can reduce the cyanide residue in cassava food products.

Key words: Linamarin, linamarase, hydrogen cyanide, cellulases, cassava slurry

Received: July 23, 2017 Accepted: November 02, 2017 Published: November 15, 2017

Citation: Maherawati, Muhammad Nur Cahyanto, Yudi Pranoto and Tyas Utami, 2017. Effect of cellulase addition on linamarin hydrolysis in cassava (*Manihot esculenta*) slurry. Pak. J. Nutr., 16: 914-920.

Corresponding Author: Tyas Utami, Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Gadjah Mada University, 55281 Yogyakarta, Indonesia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz.) is an important staple food for over 500 million people in the developing countries of tropical and sub-tropical Africa, Asia and South America[1,2]. The total worldwide production of cassava in 2014 was approximately 268 million t. Indonesia is an important cassava producer, with a production of 23.43 million t and a production area of approximately 1 million ha[3]. The root of cassava contains a highly digestible starch which has important nutritional value. The advantages of cassava over other crops are the high starch yield ha^{-1} and the high tolerance to poor soils, low rainfall and high temperatures. Cassava is also tolerant to plant diseases and pests[4-6]. On other hand, cassava contains toxic compounds, i.e., cyanogenic glucosides, mainly linamarin and to a lesser extent as lotaustralin (methyl linamarin) which are distributed widely throughout the plant[7,8]. Linamarin can be hydrolysed by endogenous linamarase to form an acetone cyanohydrin, which is either spontaneously or enzymatically transformed by hydroxynitrile lyase to release free HCN. According to Nambisan[6], cassava processing, such as boiling, blanching and sun drying still causes cyanogens to be retained at approximately 20-50%. The control of linamarin hydrolysis by processing is an efficient way to reduce the cyanide residue in cassava food. A variable amount of residual cyanide in processed cassava products, such as flour, gari and cassava chips[4,9], is still found, suggesting the inefficiency of processing methods to hydrolyse all of the linamarin in cassava. The presence of cyanogenic compound in cassava food product can cause health problems, such as neurotoxic effects and konzo[10-12].

Linamarin and linamarase are present in most plant tissues, but no HCN is detected under physiological conditions, suggesting that the enzymes and their substrate exist in two different compartments[13]. Mkpong *et al.*[14] showed that linamarase activity was apoplastic and located in the cell walls; meanwhile, linamarin was localized in the symplast. Insufficient linamarin hydrolysis could be due to the lack of contact between linamarin and endogenous linamarase since they are in different cellular locations; therefore, disruption of the cell wall could provide contact between linamarin and linamarase. In other research, insufficient hydrolysis of linamarin has also been reported due to either inadequate maceration of the plant tissues or insufficient concentration of endogenous linamarase[8]. Cassava in the slurry form and adequate incubation time will improve linamarin hydrolysis.

Cassava cell walls are composed of cellulose, hemicellulose, lignin, pectin and cassava tuber mucilage[15]. A large variety of enzymes with different specificities are required to degrade all components of lignocellulose in cassava. Cellulases is the main enzyme to hydrolyse cellulose. It is generally accepted that three types of cellulases are required to hydrolyse cellulose into glucose monomers, namely, exo-1,4-β-glucanase, endo-1,4-β-glucanase and β-glucosidase. The enzymatic hydrolysis of cellulose requires the synergistic action of these various cellulases to eventually produce glucose[16-17]. Cellulases can breakdown the cassava cell wall to allow contact between linamarin and linamarase and improve the extraction of starch that is entrapped in the cellulose-hemicellulose matrix. Therefore, the aim of this study is to improve the hydrolysis of linamarin by endogenous linamarase in wet cassava slurry to reduce the cyanide residue in cassava food product based on cassava slurry.

MATERIALS AND METHODS

Materials: The cassava roots used for experimentation were 10 months old plants of the "Pandemir" variety from local farmer at Tepus, Gunung Kidul, Yogyakarta, Indonesia. The cassava outer skins (root periderm) were removed from the roots, washed and grated manually. The cell wall degrading enzyme used was Celluclast® (Sigma Aldrich, USA).

Research procedure: This study was conducted in two stages: A preliminary study and a main study. The preliminary study included the characterization of cassava slurry consisting of determining reducing sugar content, starch content, cellulose content, linamarin content, free HCN content and endogenous linamarase activity in cassava. The main study examined the addition of cellulases into cassava slurry at various concentrations and determined the change of component in cassava slurry and microscopic structure of the cassava cell.

Characterization of cassava slurry: The grated cassava was added with water to obtain cassava slurry concentration of 50% (w/v). The cassava slurry was characterized by determining the starch content by acid hydrolysis[18], determining the cellulose content according to Chesson[19], determining the linamarin content using picrate paper kit (ANU, Australia) according to Haque and Bradbury[20] and determining the free HCN content according to William and Edwards[21]. The endogenous linamarase in cassava parenchyma was extracted and its activity was determined according to Sornyotha *et al.*[22] and Nwokoro and Anya[23] with some modifications. The fresh tissues (50 g) of cassava were grated and homogenized with 50 mL of chilled 0.2 M phosphate buffer pH 6.8 and sonicated (Sibata SU-2TH, Japan)

for 5 min. The homogenate was filtered through Whatman paper No. 4 (Sigma Aldrich, Germany) and then the filtrate was centrifuged (Eppendorf Centrifuge 5417R, Canada) at 8000 xg for 20 min at 4°C. The supernatant was collected as crude linamarase and its activity was determined. The crude linamarase was added into linamarin solution at equal volume then incubated at 50°C for 20 min. The reaction was terminated by adding 2 mL of 4% TCA and then the HCN content was determined. One unit of enzyme activity (U) was defined as the amount of enzyme that released 1 µmol of HCN per minute under the assay conditions.

Addition of cellulases in cassava slurry: Grated fresh cassava (20 g) was slurried in water until the volume was 40 mL and then the pH was adjusted to 5.5 (Ohaus ST20, Melrose, USA). The cellulose activity of celluclast was determined according to Ghose[24]. Cellulase was added into cassava slurry at 0.075, 0.15 and 0.3 FPU mL^{-1} slurry then mixed and incubated at 50°C for 24 h in a water bath (Sibata W5-240, Japan). Samples were taken at 6, 12, 18 and 24 h of incubation. The samples were filtered using a filter cloth to obtain the filtrate and then analyzed for starch release, reducing sugar and free HCN contents. The change of cell wall structure and starch granules conditions before and after selecting the enzymatic treatment were evaluated using microscopic examination.

Microscopic examination: Microscopic examination of cassava slurry was carried out before and after enzymatic treatment using light microscopy (Optical Microscope Olympus CX21 FS2, Japan) to obtain information on the cell wall structure and starch granules conditions. The samples were stained with 1% Congo red solution (Sigma Aldrich, USA). Scanning electron microscopy (SEM JSM-6510 Jeol, Japan) was used to identify the condition of the cell wall structure in more detail. Cassava slurry was previously placed on a sample holder and then dried using vacuum drying at ambient temperature for 1 h. The samples were coated with platinum and examined and photographed using SEM at an accelerating potential of 10 kV.

Statistical analysis: Three replicates were done for each treatment and the research design utilized two batches. Statistical analysis was done for all parameters using one way analysis of variance (ANOVA) for comparison of mean values among different treatments using the statistical analysis software (SAS Institute, USA). Fisher's test was performed to determine any significant differences ($p<0.05$) between different treatments. Each mean value was assigned with a superscript and a pair of mean values containing the same letter in the superscript was not significantly different.

RESULTS AND DISCUSSION

Characteristics of cassava slurry: The characteristics of cassava slurry were determined to identify the initial substances in the cassava slurry before enzymatic treatment. The starch content in cassava slurry showed that the total starch that could be extracted from cassava was 13.52 g/100 mL slurry or 71.16 g/100 g cassava dry weight (Table 1). Cassava is a physiological energy reserve with high carbohydrate content (80-90% dry basis) and starch makes up 80% of carbohydrates[25]. Cassava also contains a small amount of monosaccharides. The initial reducing sugar content in the cassava slurry was 0.44 g/100 mL slurry. The reducing sugar content can be increased as a result of polysaccharides hydrolysis.

Cellulose is an important component in cassava because it is the largest fraction of the cassava cell wall. The cellulose content in cassava slurry was 0.69 g/100 mL slurry. Cellulose consists of glucose linked by β-1,4 linkages and is further embedded in a matrix of hemicelluloses, pectin and lignin. The cellulose chains are linked by strong hydrogen bonding which makes the cellulose chains into cellulose microfibrils, causing it to be crystalline in nature and very recalcitrant to degradation[16]. The strength of cassava cell wall is an important factor in the extraction of starch and linamarin hydrolysis. Starch granules can be extracted from the cells by disintegration of the recalcitrant cell wall. Degradation of the cassava cell walls leads to the release of entrapped starch granules and allows endogenous linamarase to make contacts with linamarin.

The total linamarin in the cassava slurry was 102.3±0.93 mg HCN eq./100 mL slurry. Naturally, linamarin would be hydrolysed by endogenous linamarase resulting in release of free cyanide. The initial free cyanide in the cassava slurry was 18.02±0.86 mg/100 mL slurry. Free cyanides from linamarin hydrolysis correlate with the physical process of the size reduction (grating) of cassava, releasing endogenous linamarase from the cell wall to contact with linamarin. There was a small amount of free cyanide in the cassava slurry because not all linamarin had been hydrolysed yet. Cassava contain cyanogenic glucoside in its tissue, mainly linamarin, which are enzymatically hydrolysed to glucose, acetone and hydrogen cyanide during cell rupture[26]. Tubers of different cultivars show large variation in the cyanoglucoside content. The variation of cyanogenic potential is much larger in root parenchyma than in the root cortex or leaves[6].

Almost all parts of cassava plants contained endogenous linamarase. Leaf, stem, cortex and cassava latex contained high levels of linamarase, while it was lower in the root parenchyma. The distribution of linamarase activity varied

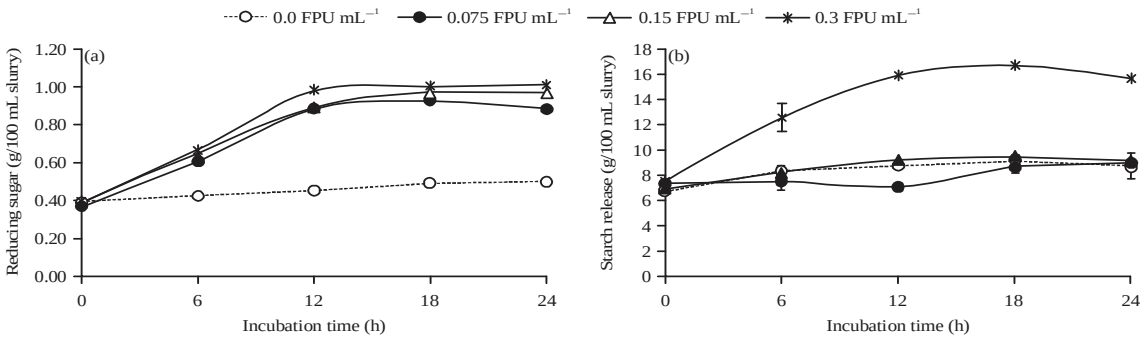

Fig. 1: (a) Reducing sugar and (b) starch release in cassava slurry during cell wall degradation by different cellulase concentrations of 0.075 (●), 0.15(Δ) and 0.3 FPU mL^{-1} (✳) and untreated cassava slurry (○) incubated at 50°C for 24 h with initial pH 5.5

Table 1: Characteristics of cassava slurry

Substances	per 100 mL slurry	per 100 g cassava (dry wt.)
Starch (g)	13.52±0.47	71.16±2.46
Reducing sugar (g)	0.44±0.003	2.30±0.018
Cellulose (g)	0.69±0.070	3.62±0.390
Linamarin (mg)	102.3±0.93	538.4±4.91
Hydrogen cyanide (mg)	18.02±0.86	23.71±1.14
Linamarase activity (U)	4.27±0.940	3.68±0.810

between different organs and tissues of the same plant and between cultivars[2,13,27]. The linamarase activity in cassava parenchyma in this study was 3.68±0.81 U g^{-1} cassava. Theoretically, these endogenous enzymes could hydrolyse all of linamarin in cassava parenchyma resulting in free HCN. However, the limited contact between linamarin and linamarase caused many linamarin to not hydrolyzed. Santana *et al.*[13] showed that no HCN was detected under physiological conditions, suggesting that the enzymes and their substrate existed in two different compartments. Degrading the cassava cell wall will rupture the barrier between linamarase and linamarin.

Effect of the addition of cellulases on reducing sugar and starch content in cassava slurry: Cellulases will catalyse the breakdown of cellulose, which is the principal framework of the cell wall, producing glucose as hydrolysis products. Ngea *et al.*[28] stated that glucose was the main constituent of cell wall material and probably originated primarily from cellulose. Determination of the reducing sugar content during incubation of cassava slurry was used to estimate the cellulase activity. The results showed that the reducing sugar increased at all cellulases concentrations suggested that degradation of cellulose in the cassava slurry occurred (Fig. 1a). There was no significant difference in the reducing sugar content in all enzyme loadings, revealing that cellulase concentrations used effectively degraded the cell wall. The enzyme loading in this study was enough to support endoglucanase activity in cellulase to generate many new reducing and non-reducing chain ends for exoglucanase attack. Van Dyk and Pletschke[16] showed that enzyme loading was one of the factors affecting the hydrolysis process.

The starch release in cassava slurry increased significantly when the addition of the highest cellulase loading (0.3 FPU mL^{-1}) reached 15.72±0.07 g/100 mL slurry (Fig. 1b). There was an enhancement of 16.44% from the initial starch content in cassava slurry. Cellulose and other polysaccharides such as hemicelluloses, lignin and pectin in the cell wall are associated as complex substances. At low enzyme loading, cellulose was not able to penetrate deeper into the cell wall structure. Disintegration of the cassava root cell walls is a multifactorial problem since the plant cell wall strength depends on the properties of its components, its composition, intra-structural interactions and the molecular mechanism of cell adhesion[29]. An adequate amount of cellulase is able to degrade the cell wall matrix and create rupture, that which is able to release the entrapped starch. The cellulase activity results in the fragmentation of the cassava cell walls, facilitating the release of starch that is entrapped in the cellulose-hemicellulose matrix[15]. The results obtained in this work indicated that cellulase plays an important role in hydrolysis of the cell walls, which results in the reducing sugar and release of more starch granules from the cell.

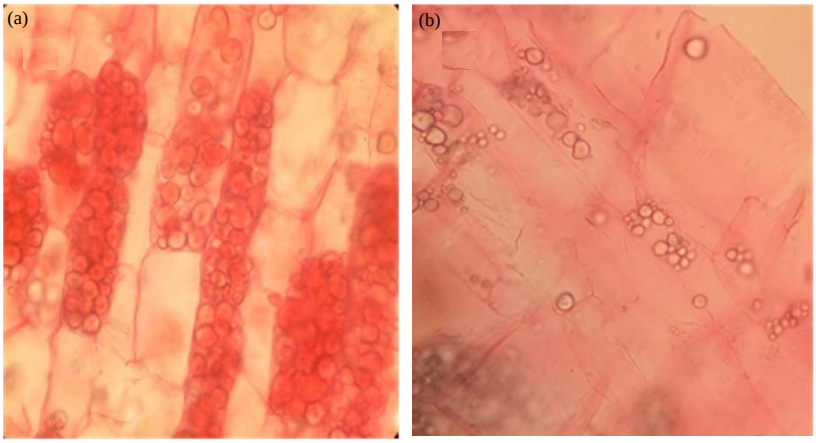

Fig. 2: Light microscopy images of the (a) native cassava parenchyma and (b) cassava slurry with the addition of 0.3 FPU mL^{-1} cellulase incubated at 50°C for 24 h

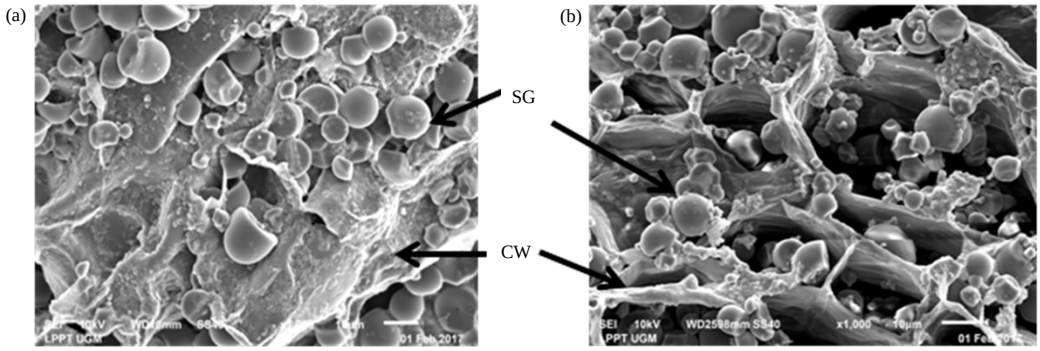

Fig. 3: Scanning electron microscopy images of the (a) native cassava parenchyma and (b) cassava slurry with the addition of 0.3 FPU mL^{-1} cellulase (b) incubated at 50°C for 24 h. SG: starch granule. CW: Cell wall

Changes in the cassava cell wall structure by cellulases: The addition of cellulases in the cassava slurry led to degradation of the cell wall due to breakdown of the glycosidic bonds at the microfibril surface; thus the cellulose chains became loosely associated with one another. The light microscopy image of cassava parenchyma from cassava slurry after 24 h of incubation with cellulase addition (0.3 FPU mL^{-1}), shown in Fig. 2, indicates that in native cassava, the cell wall structure was still intact and exhibited sharp boundaries between the cells, while in the cassava slurry with cellulase treatment, the cassava cell wall structure showed a more diffuse background and was unfilled by starch granules. This result suggested that cellulases weakened the cell walls so that starch granules can release out of the cells.

Odoch *et al.*[30] reported that exogenous cellulolytic enzymes enabled access to embedded cellulose microfibrils and caused a slight reduction in cellulose crystalline. In another study, Adetunji *et al.*[15] showed a schematic illustration that explained the cellulose activity resulting in the breakdown and hydrolysis of the cellulosic cell walls, which released entrapped starch granules. A phenomenon also occurred in this study and was confirmed by scanning electron microscopy, revealed that the native cassava cell structure was initially intact and encased by the cell wall matrix with the starch granules inside and only a few starch granules were outside (Fig. 3a). After 24 h of incubation with cellulases (0.3 FPU mL^{-1}), the cassava cell wall structure showed opening up of the cell walls. Space in the cassava cell unfilled with starch granules indicated that many starch granules were release from the cell (Fig. 3b). The activities of cellulases acting on the cell wall matrix led to a change of the structural and composition of the cassava parenchyma cell wall. The cellulose activity resulted in the fragmentation and hydrolysis of the cellulosic cell walls, thus releasing the trapped starch granules[15].

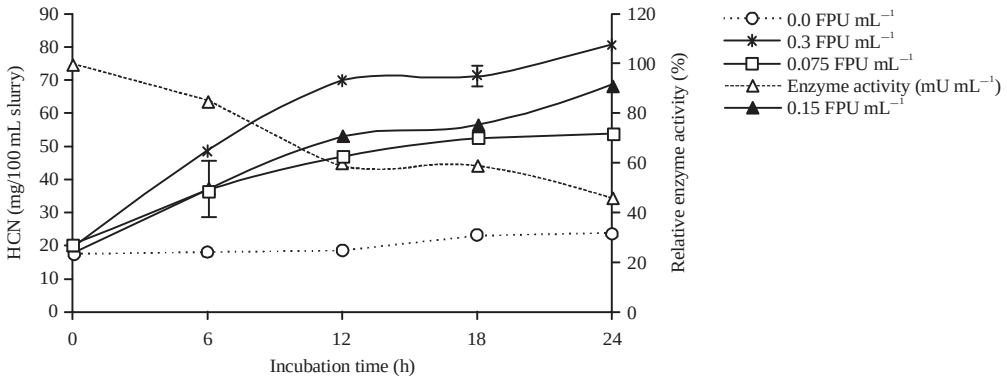

Fig. 4: HCN content in cassava slurry during cell wall degradation by different cellulase concentrations of 0.075 (□), 0.15 (▲), 0.3 FPU mL^{-1} (✳) and untreated cassava slurry (○) and enzyme activity (Δ) incubated at 50°C for 24 h with initial pH 5.5

Effect of cassava cell wall degradation by cellulases on HCN content in cassava slurry: The HCN content in cassava slurry was a result of linamarin hydrolysis by endogenous linamarase in the cassava root. Linamarin hydrolysis requires a trigger to breakdown the cell wall. Cellulase activity will increase the degradation of cassava cell walls to sufficiently release endogenous linamarase to catalyse linamarin. This result showed that the addition of cellulase at all enzymes loadings significantly increased the HCN content in cassava slurry (Fig. 4). Increasing the cellulase concentrations caused more rupture on the cell wall leading to greater liberation of linamarase that hydrolysed linamarin. The HCN content increased significantly during 12 h of incubation which then became slower until the end of incubation. This effect was corresponded to the endogenous linamarase activity in cassava. The enzyme activity assay of endogenous linamarase in cassava slurry revealed that enzyme activity could be maintained at 59% after 12 h of incubation and 46% after 24 h of incubation. Hence, the linamarase activity to catalysing linamarin became slower after 12 h. The free HCN content in cassava slurry after incubation with cellulases (0.3 FPU mL^{-1}) at 50°C for 24 h was 80.97±0.92 mg/100 mL slurry. This suggested that approximately 79% of linamarin in the cassava slurry was hydrolysed. Endogenous linamarase in cassava itself only hydrolysed 19% of linamarin. The cellulases succeeded in disrupting the cassava cell walls and facilitating contact between linamarin and endogenous linamarase, which resulted in improvement of free HCN in cassava slurry. This study is part of a major study on cyanide detoxification in cassava slurry by the enzymatic method. The implication of this study is that the cellulases concentration increases the linamarin hydrolysis in cassava slurry. This study is recommended to the cassava industry that requires low-cyanide cassava slurry for further processing.

CONCLUSION

The treatment of cassava slurry with cellulases not only can enhance the reducing sugar and the release of starch entrapped in the cell wall matrix, but it can also improve the liberation of linamarase that hydrolyse linamarin. Degradation of the cell wall by cellulases (0.3 FPU mL^{-1}) increased the HCN content to 80.97±0.92 mg/100 mL slurry at 24 h of incubation. This was approximately 79% of linamarin in the cassava slurry that became hydrolyzed, resulting in free cyanide. The breakdown of the cassava cell wall structure and the release of starch granules from the cells were shown by light microscopy and SEM. Endogenous linamarase in cassava itself was not able to hydrolyse all of the linamarin in cassava. The addition of cellulases in cassava slurry degraded the cell walls thus allowing contact between linamarase and linamarin and producing HCN.

SIGNIFICANCE STATEMENT

This study discovered the effect of cellulase addition which can be beneficial for linamarin hydrolysis in cassava slurry. This study can help researchers to uncover the critical area of cyanide detoxification in cassava processing that many researchers were not able to explore. Thus, a new theory on adding cellulose to increase linamarin hydrolysis, may be arrived at.

REFERENCES

1. El-Sharkawy, M.A., 2003. Cassava biology and physiology. Plant Mol. Biol., 53: 621-641.
2. Nambisan, B. and S. Sundaresan, 1994. Distribution of linamarin and its metabolising enzymes in cassava tissues. J. Sci. Food Agric., 66: 503-507.

3. FAO., 2017. FAOSTAT agricultural database. FAO., Rome, Italy. http://www.fao.org/faostat/en/#search/cassava
4. Burns, A.E., R.M. Gleadow, A.M. Zacarias, C.E. Cuambe, R.E. Miller and T.R. Cavagnaro, 2012. Variations in the chemical composition of cassava (*Manihot esculenta* Crantz) leaves and roots as affected by genotypic and environmental variation. J. Agric. Food Chem., 60: 4946-4956.
5. Shanavas, S., G. Padmaja, S.N. Moorthy, M.S. Sajeev and J.T. Sheriff, 2011. Process optimization for bioethanol production from cassava starch using novel eco-friendly enzymes. Biomass Bioenergy, 35: 901-909.
6. Nambisan, B., 2011. Strategies for elimination of cyanogens from cassava for reducing toxicity and improving food safety. Food Chem. Toxicol., 49: 690-693.
7. Cardoso, A.P., E. Mirione, M. Ernesto, F. Massaza, J. Cliff, M.R. Haque and J.H. Bradbury, 2005. Processing of cassava roots to remove cyanogens. J. Food Compos. Anal., 18: 451-460.
8. Petruccioli, M., L. Brimer, A.R. Cicalini and F. Federici, 1999. The linamarase of *Mucor circinelloides* LU M40 and its detoxifying activity on cassava. J. Applied Microbiol., 86: 302-310.
9. Yeoh, H.H. and F. Sun, 2001. Assessing cyanogen content in cassava-based food using the enzyme-dipstick method. Food Chem. Toxicol., 39: 649-653.
10. Adamolekun, B., 2010. Etiology of Konzo, epidemic spastic paraparesis associated with cyanogenic glycosides in cassava: Role of thiamine deficiency? J. Neurol. Sci., 296: 30-33.
11. Rivadeneyra-Dominguez, E., A. Vazquez-Luna, J.F. Rodriguez-Landa and R. Diaz-Sobac, 2013. Neurotoxic effect of linamarin in rats associated with cassava (*Manihot esculenta* Crantz) consumption. Food Chem. Toxicol., 59: 230-235.
12. Tylleskar, T., M. Banea, N. Bikangi, R.D. Cooke, N.H. Poulter and H. Rosling, 1992. Cassava cyanogens and konzo, an upper motoneuron disease found in Africa. Lancet, 339: 208-211.
13. Santana, M.A., V. Vasquez, J. Matehus and R.R. Aldao, 2002. Linamarase expression in cassava cultivars with roots of low- and high-cyanide content. Plant Physiol., 129: 1686-1694.
14. Mkpong, O.E., H. Yan, G. Chism and R.T. Sayre, 1990. Purification, characterization and localization of linamarase in cassava. Plant Physiol., 93: 176-181.
15. Adetunji, A.I., H. du Clou, S.N. Walford and J.R. Taylor, 2016. Complementary effects of cell wall degrading enzymes together with lactic acid fermentation on cassava tuber cell wall breakdown. Ind. Crops Prod., 90: 110-117.
16. Van Dyk, J.S. and B.I. Pletschke, 2012. A review of lignocellulose bioconversion using enzymatic hydrolysis and synergistic cooperation between enzymes-factors affecting enzymes, conversion and synergy. Biotechnol. Adv., 30: 1458-1480.
17. Hall, M., P. Bansal, J.H. Lee, M.J. Realff and A.S. Bommarius, 2011. Biological pretreatment of cellulose: Enhancing enzymatic hydrolysis rate using cellulose-binding domains from cellulases. Bioresour. Technol., 102: 2910-2915.
18. AOAC., 1984. Official Methods of Analysis. 14th Edn., Association of Official Agricultural Chemists, Washington, DC., USA.
19. Chesson, A., 1981. Effects of sodium hydroxide on cereal straws in relation to the enhanced degradation of structural polysaccharides by rumen microorganisms. J. Sci. Food Agric., 32: 745-758.
20. Haque, M.R. and J.H. Bradbury, 2004. Preparation of linamarin from cassava leaves for use in a cassava cyanide kit. Food Chem., 85: 27-29.
21. Williams, H.J. and T.G. Edwards, 1980. Estimation of cyanide with alkaline picrate. J. Sci. Food Agric., 31: 15-22.
22. Sornyotha, S., K.L. Kyu and K. Ratanakhanokchai, 2010. An efficient treatment for detoxification process of cassava starch by plant cell wall-degrading enzymes. J. Biosci. Bioeng., 109: 9-14.
23. Nwokoro, O. and F.O. Anya, 2011. Linamarase enzyme from *Lactobacillus delbrueckii* NRRL B-763: Purification and some properties of a β-glucosidase. J. Mexican Chem. Soc., 55: 246-250.
24. Ghose, T.K., 1987. Measurement of cellulase activities. Pure Applied Chem., 59: 257-268.
25. Montagnac, J.A., C.R. Davis and S.A. Tanumihardjo, 2009. Processing techniques to reduce toxicity and antinutrients of cassava for use as a staple food. Comprehen. Rev. Food. Sci. Food Saf., 8: 17-27.
26. Tivana, L.D., J.D.C. Francisco, F. Zelder, B. Bergenstahl and P. Dejmek, 2014. Straightforward rapid spectrophotometric quantification of total cyanogenic glycosides in fresh and processed cassava products. Food Chem., 158: 20-27.
27. Nambisan, B., 1999. Cassava latex as a source of linamarase for determination of linamarin. J. Agric. Food. Chem., 47: 372-373.
28. Ngea, G.L.N., F. Guillon, J.J.E. Ngang, E. Bonnin, B. Bouchet and L. Saulnier, 2016. Modification of cell wall polysaccharides during retting of cassava roots. Food Chem., 213: 402-409.
29. Favaro, S.P., A. Beleia, N. da Silva Fonseca Jr. and K.W. Waldron, 2008. The roles of cell wall polymers and intracellular components in the thermal softening of cassava roots. Food Chem., 108: 220-227.
30. Odoch, M., E.M. Buys and J.R.N. Taylor, 2017. Mechanism of cassava tuber cell wall weakening by dilute sodium hydroxide steeping. Food Chem., 228: 338-347.