

# ENZYMATIC HYDROLYSIS OF BITTER CASSAVA AND GADUNG STARCHES WITH DIFFERENT COMPOSITIONS AT LOW TEMPERATURE

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**Abstract.** The effect of compositions of bitter cassava (*Manihot glaziovii*) and *gadung* (*Dioscorea hispida* Dennst) starches on reducing sugar during hydrolysis using granular starch hydrolyzing enzyme (GSHE) was studied. All hydrolyses were conducted at concentration of substrate was 200 g.L<sup>-1</sup>, while concentration of enzyme was 1.5 % (w/w), during of hydrolysis time 24 h, at 30°C. Mass composition of bitter cassava and *gadung* starches were 9:1 to 1:9. The increase *gadung* starch compositions will decrease the reducing sugar. The optimum condition of the process using concentration of substrate 200 g.L<sup>-1</sup> with compositions of bitter cassava and *gadung* starches was 9:1 at 18 h. It was found that reducing sugar was 50.20 g.L<sup>-1</sup>. The concentration of reducing sugar mainly depend on starch content on bitter cassava, it is much bigger than the *gadung* starch.

## 1 Introduction

In Indonesia, bitter cassava (*Manihot glaziovii*) and bitter yam (*Dioscoreae hispida* Dennst) which locally known as *gadung* are commonly found in secondary forest. They grow under shaded areas or near streams, not consumed by humans because of the presence of poisonous alkaloids known as cyanogens and dioscorin [1], which can seriously impact the health of the people who consume the tuber. Lambri et al. [2] reported that the total cyanide content of cassava root for sweet white and bitter white were 374 and 442 mg/kg (d.w), respectively. Djazuli and Bradbury [3] reported of 14 samples of cyanogen content of cassava starch in Indonesia. The maximum value of cyanogens content in cassava starch and cassava flour were 12 ppm and 149 ppm, respectively.

The conventional conversion of starch to glucose requires a two-step process, namely, liquification and saccharification. During liquefaction, gelatination of starch is promoted by applying high temperature (90-100 °C),  $\alpha$ -amylase is added in order to convert starch into dextrans, maltose and maltotriose. The resulting mash is then cooled to 60 °C and glucoamylase is added to convert dextrans to fermentable sugars as glucose [4-7]. Granular starch hydrolyzing enzyme (GSHE) is a mixture of  $\alpha$ -amylase and glucoamylase which hydrolyzes granular starch directly into fermentable sugars and works at low temperature (30 to 48°C) and pH (4.0 to 4.5) [8]. This process is an energy-intensive, and therefore increases the production cost. It has been estimated that about 30-40% of the total energy is demanded during ethanol production from starch [5]. The use of GSHE presents other advantages, as the GSHE will negate liquefaction and saccharification.

Previous researches were commonly conducted via conventional hydrolysis and hydrolysis without heating a single substrate, for example the hydrolysis of corn, wheat, tapioca, sago, sorghum starches, etc, only few researches were conducted on hydrolysis enzymatic of double substrate. Uthumporn et al. [9] also reported the hydrolysis of maize, sago and sweet potato starches using  $\alpha$ -amylase and glucoamylase, at 35°C to 50°C. Li et al. [10] reported the hydrolysis of corn and triticale starches at 30-50°C using  $\alpha$ -amylase and glucoamylase. Shariffa [11] investigated hydrolysis of tapioca and sweet potato starches by  $\alpha$ -amylase and glucoamylase, at 35-65°C.

The objective of this study is to investigate the effect of bitter cassava and *gadung* starches compositions and on reducing sugar.

## 2 Materials and method

### 2.1 Cassava and *gadung* tubers

Ten month old of bitter cassava was called Pandemir (*Manihot glaziovii*) tuber was obtained from Wonogiri district in Indonesia, while *gadung* tuber with 9 month old was obtained in Godean district in Indonesia.

The extraction method applied to the *gadung* is the same as the bitter cassava extraction method. The method of extraction of the bitter cassava starch, physicochemical properties of the bitter cassava and *gadung* used in this study were the same as the those used in the previous research conducted by Hargono [12]. Potassium sodium tartrate tetrahydrate and 3,5-Dinitrosalicylic acid, sodium hydroxide (98%, Merck), sodium sulfite

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(98.5%, Merck), sulfuric acid (98.5%, Merck), sodium acetate buffer, glucose (99.5%), were purchased from their authorized distributor. Granular starch hydrolyzing enzyme as Stargen™ 002, which is a mixture of  $\alpha$ -amylase and glucoamylase was produced by Genencor (Palo Alto, USA). The activity and optimal pH range declared by the producer are 570 GAU/g and 4.0-4.5, respectively [8].

## 2.2 Enzymatic hydrolysis

All hydrolyses were conducted at concentration of starch slurry of 200 g.L<sup>-1</sup>. Mass compositions of bitter cassava and *gadung* starches were 9:1, 4:1, 2.33:1, 1.5:1, 1.25:1, 1:1, 1:9, 1:4, 1:2.33 and 1:1.5. Concentration of Stargen™ 002 was 1.5 % (w/w) was added into the samples. Samples were then incubated in an incubator shaker at 30±1°C, speed of 110 rpm. After 24 h, hydrolysis was stopped by adjusting the pH 4 (in 50 mM sodium acetate buffer). Samples were periodically withdrawn from the flask at 6 h interval and substantially subjected to reducing sugar analysis. Before the samples were analyzed by Spectrophotometer, they were centrifuged (100Hz, 4°C and 10 min) to obtain the filtrate. All experiments were performed in triplicates.

## 2.3 Analytical methods

The starch content was determined by AOAC method [13]. The water content in cassava starch was determined by standard drying method in oven at 105 °C to constant mass [14]. The total cyanide analysis by the acid hydrolysis method [15]. During the cassava starch hydrolysis, the content of reducing sugar was measured using dinitrosalicylic acid method [16]. Reagent consisting of aqueous solution of 1% 3,5-dinitrosalicylic acid, 0.05% Na sulfit, 20% Na-K tartrate and 1% NaOH solution was added in the ratio 3:1 to the samples in glass tubes, shaken in incubated in a boiling water bath for 8 min. Reacted samples were cooled in an ice water bath for 5 min, prior to measuring absorbance at 540 nm by using a UV/visible spectrophotometer (UV-160A, SHIMADZU, Kyoto, Japan). Glucose (0 to 60 g.L<sup>-1</sup>) was used as standard, therefore reducing sugar concentrations was reported as g.L<sup>-1</sup>.

## 3 Result and discussion

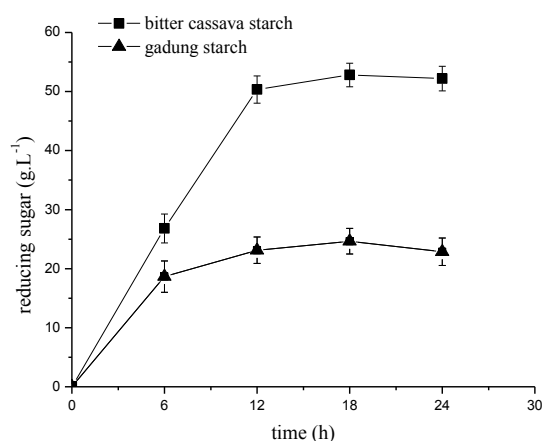
### 3.1 Determination of reducing sugar from bitter cassava and *gadung* starches hydrolysis

The reducing sugars obtained from bitter cassava and *gadung* starches by Stargen™ 002 with concentration 1.5 % (w/w), and concentration of starch 200 g.L<sup>-1</sup>, at 30°C and pH 4, during of hydrolysing time 0 to 24 hours are given in Figure 1. Over the hydrolysis time from 0 to 24 h, product of the reducing sugar obtained from of bitter cassava starch was higher than *gadung* starch. Production hydrolysis from 0-18 h showed a maximum reducing sugar concentrations (bitter cassava starch

52.80 g.L<sup>-1</sup> and *gadung* starch 24.65 g.L<sup>-1</sup>), while production rate showed of bitter cassava starch 2.93 g.(Lh)<sup>-1</sup> and *gadung* starch 1.37 g.(Lh)<sup>-1</sup>, respectively. Furthermore after 18 hours, the hydrolysis of both of cassava and *gadung* starches decelerated, and then followed by constant rate to 24 h. The differences of the reducing sugar concentrations, it is because the starch content in *gadung* is much lower than the bitter cassava starch, as shown in Table 1 and so probably amylose content in bitter cassava starch is much lower than *gadung* starch. Sharma et al. [17] investigated effect amylose:amylopectin ratios on ethanol production from maize starch using GSHE. They concluded the amylose content in starch increased, the amount of ethanol produced decreased. Wu et al. [18] reported the result showed that the amylose content in starch had a significant effect on the ethanol conversion efficiency. Conversion efficiency increased as the amylose content decreased. So in addition the cyanide content in *gadung starch* is much higher than bitter cassava starch (Table 1), as a consequence the cyanide inhibit the enzyme leading to decrease of enzyme activity.

**Table 1.** Properties of bitter cassava and *gadung* starches

Parameters	bitter cassava starch	<i>gadung</i> starch
Moisture content (%), w/w	10.80	11.12
Starch (%), w/w	82.70	78.85
Cyanide, mg/kg	152.45	186.82



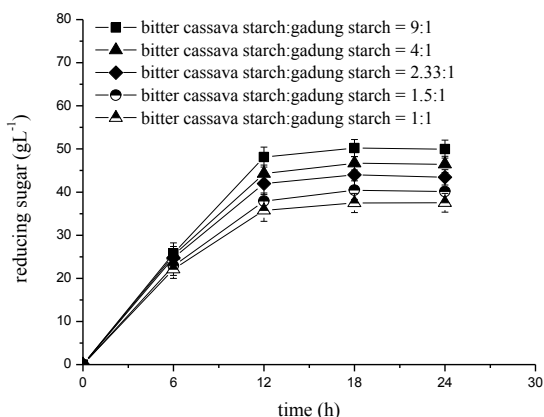
**Fig 1.** Hydrolysis profile of bitter cassava and *gadung* starches at concentration of substrate 200 g.L<sup>-1</sup>, concentration of Stargen™ 002 1.5 % (w/w), pH 4 and 30°C. Each value represents the mean of three independent measurements. Deviation from the mean below 5% for all values displayed.

Hargono et al. [12] investigated of effect of cyanide on reducing sugar from bitter cassava and *gadung* flours, we concluded increasing of cyanide concentration during hydrolysis process, decreased enzyme activity, as a consequence amount of reducing sugar decreased. Shanavas et al. [19] reported the reducing sugar formed from cassava starch by varying

level of Stargen, at pH 4.5 and 30°C, it was found as 98.3 g/L could be hydrolyzed by Stargen level 100 mg on 10% (w/v) starch. Also reported by Yussof et al. [20], that the during time 8 to 24 h hydrolysis native tapioca starch was increasing reducing sugar, as indicated by increasing of dextrose equivalent (DE) 18 to 35.7%.

### 3.2 Effect of different composition bitter cassava and gadung starches on reducing sugar

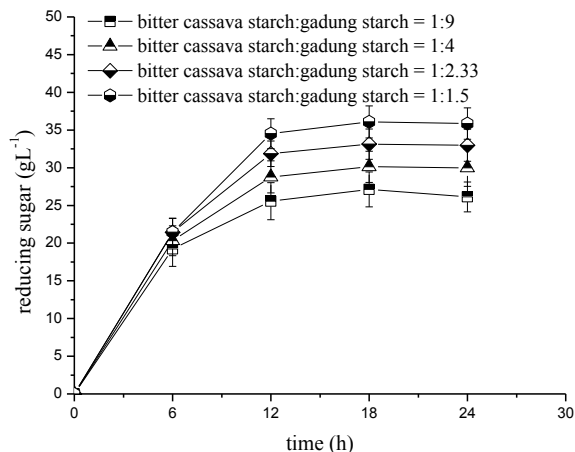
Figure 2 shows reducing sugar release by enzymatic hydrolysis at a substrate sugar concentration 200 g.L<sup>-1</sup>, concentration Stargen™ 002 was 1.5% (w/w), 30°C and pH 4 at various compositions of bitter cassava to *gadung* starches (9:1 to 1:1) during 24 h. It was found the maximum of concentration of reducing sugars at 18 h were 50.20 g.L<sup>-1</sup>, (9 :1), 46.68 g.L<sup>-1</sup> (4:1), 43.98 g.L<sup>-1</sup> (2.33:1), 40.45 g.L<sup>-1</sup> (1.5:1), and 47.49 g.L<sup>-1</sup> (1 :1). The production of reducing sugar increased when bitter cassava to *gadung* starches ratio in the starch composition increased.



**Fig 2.** Hydrolysis profile of bitter cassava and *gadung* starches compositions (9 :1 to 1:1) w/w, at concentration of substrate 200 g.L<sup>-1</sup>, concentration of Stargen™ 002 1.5 % (w/w), pH 4 and 30°C. Each value represents the mean of three independent measurements. Deviation from the mean below 5% for all values displayed.

The result showed that reducing sugar mainly depend on starch content on bitter cassava, it is much bigger than the *gadung* starch. Thereafter the reducing sugar release by enzymatic hydrolysis at a substrate concentration 200 g.L<sup>-1</sup>, concentration Stargen™ 002 was 1.5% (w/w), 30°C and pH 4 during 24 h at various compositions of bitter cassava to *gadung* starches from 1:9 to 2:3, as shown in Figure 3. The maximum reducing sugar release at 18 h. It was 27.12 g.L<sup>-1</sup> (1:9), 30.12 g.L<sup>-1</sup>, (1:4), 33.14 g.L<sup>-1</sup> (1:2.33) and 36.08 g.L<sup>-1</sup> (1:1.5). Masiero et al. [21] reported a hydrolysis of fresh potato (200 g.L<sup>-1</sup>) with Stargen™ 002 (45 GAU.g<sup>-1</sup>) during 62 h. After the first 20 h, glucose was liberated 30 g.L<sup>-1</sup>. Shanavas et al. [18] investigated a hydrolysis of cassava starch (200 g.L<sup>-1</sup>), by Stargen™ 002 with concentration 0.4 % (w/w) at 24 h and 30°C, reducing sugar was

obtained 185 g.L<sup>-1</sup>. The difference in hydrolysis time of our study are much shorter compared to other researchers, because the enzyme concentration is much bigger so that it is more active in degrading starch to reducing sugar.



**Fig 3.** Hydrolysis profile of bitter cassava and *gadung* starches compositions (1:9 to 1:1.5) w/w, at concentration of substrate 200 g.L<sup>-1</sup>, concentration of Stargen™ 002 1.5 % (w/w), pH 4 and 30°C. Each value represents the mean of three independent measurements. Deviation from the mean below 5% for all values displayed

### Conclusion

The experiments conducted an attempt is made to present kinetic expression for the hydrolysis of bitter cassava and *gadung* starches compositions, at concentration of substrate 200 g.L<sup>-1</sup>, concentration of Stargen™ 002 1.5 % (w/w), pH 4 and 30 °C. The results showed that at various compositions of bitter cassava to *gadung* starches (9:1 to 1:9) during 24 h. It was found the maximum of concentration of reducing sugars at 18 h at ratio 9:1 was 50.20 g.L<sup>-1</sup>, while the minimum of concentration of reducing sugars at 18 h at ratio 1:9 was 27.12 g.L<sup>-1</sup>. The release of reducing sugar increased when bitter cassava to *gadung* starches compositions increased, the concentration of reducing sugar mainly depend on starch content on bitter cassava, it is much bigger than the *gadung* starch.

### References

1. M. Nashriyah, Y. Nornasuha, T. Salmah, N. Norhayati, M. Rohaizad, (2010) *Bulletin UniSZA*, **4** (2010)
2. M. Lambri, M.D. Fumi, A.Roda, D.M. Faveri, Afr J Biotechno. **19** (2013)
3. M. Djazuli, J.H. Bradbury, Food Chem. **65** (1999)
4. R.F. Power, *The Alcohol Textbook*, 4<sup>th</sup> edition, 23-32, (Nottingham University Press, Nottingham, 2003)

5. G.H. Robertson, D.W.S., Wong, C.C. Lee, J.Agric. Food. Chem. **54** (2006)
6. E. Sarikaya, T. Higasa, M. Adachi, B. Mikami, Process Biochem. **35** (2005)
7. S. Govindasamy, C.G. H.A. Oates, Carbohydr. Polym. **18** (1992)
8. Genencor (2009) STARGEN™ 002: granular starch hydrolyzing enzyme for ethanol production
9. U. Uthumporn, Y.N. Shariffa, A.A. Karim, Appl. Biochem. Biotechnol. **166** (5) (2012)
10. J. Li, T. Vasanthan, D.C. Bressler, Carbohydr. Polym. **87** (2), (2012)
11. Y.N. Sharrifa, A.A. Karim, A. Fazilah, I.S.M. Zaidul Food Hydrocoll. **23**, (2009)
12. H. Hargono, B. Jos, A.C. Kumoro, Bull. Chem. React. Eng. Catal. **12** (2) (2017)
13. AOAC. Official Methods of Analysis of AOAC Intl. 16<sup>th</sup> ed. Method 991.43. Association of Official Analytical Chemists, Arlington, VA, USA. (1995)
14. B.V. Mc Cleary, V. Solah, T.S. Gibson, J.Sereal Sci. **20** (1994)
15. J.H. Bradbury, S.V. Egan, M.J. Lynch, J. Sci. Food. Agr. **55** (1991)
16. G.L. Miller G.L. Anal.Chem. **31** (1959)
17. V. Sharma, K.D. Rausch, M.E. Tumbleson, V. Singh, Starch/Starke **59** (2007)
18. X. Wu, R. Zhao, D. wang, S.R. Bean, P.A. Seib, M.R. Tuinstra, M. Cambell, A. O Brien, Cereal Chem. **63** (5) (2006)
19. S. Shanavas, G. Padmaja, S.N. Moorthy, M.S. Sajeev, J.T. Sheriff, Biomass and Bioenergy **35** (2) (2010)
20. N.S.Yussof, U.Utra, A.K. Alias, Starch/Starke **65** (2013)
21. S.S. Masiero, A. Peretti, L.F. Trierweiler, J.O. Trierweiler, Biomass and Bioenergy **70** (2014)