Effect of isoamylase application on chemical characteristic of cassava root meal starch

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Abstract. Cassava root meal (CRM) is still rarely used as an ingredient for poultry feed. The high viscosity component can be reduced by enzymatic hydrolysis. However, there is limited information about enzyme treatment on cassava root meal. The objective was to identify and evaluate the effect of isoamylase hydrolysis on the chemical characteristic of hydrolyzed cassava root meal starch which has amylose/amylopectin ratio approached the corn. The cassava root meal was weighed each 200 g containing five levels of isoamylase (CRM-A) 0.01%, (CRM-B) 0.02%, (CRM-C) 0.03%, (CRM-D) 0.04%, (CRM-E) 0.05% (w/w) were moistened with 135 ml distilled water and incubated at 50 °C for 12 hours. Total starch, amylose, amylopectin, total sugar, and reducing sugar content of hydrolyzed product were measured. The result showed that there were increasing of total starch and amylose content of hydrolyzed cassava root meal and decreasing in amylopectin content and amylose/amylopectin ratio. Reducing sugar increased along with increasing enzyme addition 1.81 – 3.07% DM which corresponds to degree of hydrolysis to 54.52 – 72.99%. It can be concluded that the different levels of isoamylase addition will produce cassava root meal starch with different chemical characteristics. The addition 0.05% isoamylase is able to make amylose/amylopectin ratio of hydrolyzed cassava root meal approached the corn.

1. Introduction

Corn is one of the most important commodity also the main feed ingredient of poultry farm and become raw material of processed industry. The rapid development of the poultry industry will encourage the increasing demand for corn. Rising of raw material costs have accelerated the demand for finding alternative feed ingredients that can replace this material with lower production costs. Cassava is the highest source of carbohydrate among staple crops and can potentially replace corn as a source of energy in poultry feed. However, cassava also has a disadvantage that is high levels of resistant starch and amylopectin content. Resistant starch can not be digested and utilized by poultry. The main composition of cassava starch generally consists of amylose and amylopectin. Cassava starch contains 17% amylose and 83% amylopectin, compared to corn starch which has 28% amylose and 72% amylopectin [1-2]. High levels of starch resistance in cassava were probably 82.85% amylopectin with 5.79% of branch association...
and amylose 17.25% with 0.48% branch association [3]. Another possible explanation is amylopectin in cassava has a relatively long chain length. Starch is degraded by amylases, of which isoamylase (glycogen-6-glucanohydrolase, E.C.3.2.1.68) also known as debranching enzyme hydrolases 1, 6-α-D-glycosidic linkages of glycogen, amylopectin and α and β limit dextrins, producing linear maltooligosaccharides [4]. Isoamylase is used primarily in the production of food ingredients from starch like glucose, maltose, trehalose and cyclodextrins [5]. An understanding of the relationship between enzyme levels and chemical characteristic of hydrolyzed products is essential for using in the poultry feed industry. This study was designed to identify and evaluate the effect of isoamylase hydrolysis on the chemical characteristic of hydrolyzed cassava root meal starch which has amylose/amylopectin ratio approached the corn.

2. Material and method

2.1. Material
Cassava root meal was obtained from UD. Setia Flour Product (Cikarawang, Bogor). Isoamylase (Food Grade) was provided from Creative Enzymes (USA), Creative Biomart, Inc. This enzyme has optimum activity at a pH around 5.6 – 7.0 and at temperature between 50 – 55 °C. The enzyme activity ≥ 10,000 U/g.

2.2. Enzyme hydrolysis
The cassava root meal (CRM) was weighed each 200 g containing five levels isoamylase (CRM-A) 0.01%, (CRM-B) 0.02%, (CRM-C) 0.03%, (CRM-D) 0.04%, (CRM-E) 0.05% (w/w) and wetted with 135 ml of distilled water. The mixtures were incubated in waterbath at 50 °C for 12 hours. After that, the samples were dried in oven at 60 °C for 24 hours to stop the enzyme activity.

The moisture content were determined by gravimetric methods [6]. Total starch were analysed using calorimetric method [7] and amylose content were analysed by spectrophotometric [8]. Amylopectin content was calculated by difference between total starch and amylose content. The degree of hydrolysis was measured as an increase in the content of reducing sugars. It was measured using Luff Schrool method [9].

2.3. Data analysis
All chemical analyses were performed in two replicates and the result were expressed as the mean ± standard deviation (SD).

3. Result and discussion
The data presented in Table 1 showed the comparison of starch content in corn and native cassava as a control and hydrolyzed cassava root meal. All hydrolyzed cassava root meal showed increasing in total starch and amylose content and tended to decrease amylopectin content as the increasing of isoamylase addition. Cassava root meal with the addition of 0.05% isoamylase (CRM-E) produces 64.27% amylopectin content which is almost closely to corn 57.41% and has the lowest amylose / amylopectin ratio 1:2.63. Hydrolysis of starch by enzyme is influenced by enzyme diffusion rate, substrate accessibility, substrate recognition leading to the formation of enzyme substrate complex and appropriate catalytic action [10].

Similarly, the long incubation time required to achieve complete hydrolysis is an indicator for the slow rate of enzyme diffusion imposed by highly viscous cassava gel. While isoamylase capable of hydrolyzing the bond of 1, 6-α-D-glycosidic of amylopectin [11]. This can be seen with increasing addition of isoamylase tends to decrease amylopectin content in cassava root meal in order to lower amylose/amylopectin ratio.
The data presented in Table 2 revealed the degree of hydrolysis by isoamylase in cassava root meal. The total sugar of hydrolyzed cassava root meal ranged from 3.31 – 4.20% DM and reducing sugar ranged between 1.18 – 3.07% DM which correspond to degree of hydrolysis 54.52 – 72.99%. The low reducing sugar value of cassava starch implies an abundance of amylopectin and amylose polymers that are not degraded in starch [12]. On the contrary the result showed that reducing sugar value in hydrolyzed cassava root meal tended to increase with the increasing of isoamylase compared to native cassava which is the total sugar 3.28% DM and reducing sugar 1.04% DM corresponds to a degree of hydrolysis only 31.77%.

### Table 1. Total starch, amylose, and amylopectin content of corn and cassava root meal

| Sample   | Total Starch (% DM) | Amylose (% DM) | Amylopectin (% DM) | Amylose/Amylopectin Ratio |
|----------|---------------------|----------------|---------------------|---------------------------|
| Corn     | 85.30 ±0.18         | 27.89 ±0.03    | 57.41 ±0.16         | 1:2.05                    |
| Cassava  | 85.78 ±0.28         | 20.52 ±0.03    | 65.26 ±0.31         | 1:3.18                    |
| CRM-A    | 85.47 ±0.07         | 20.55 ±0.06    | 64.92 ±0.01         | 1:3.16                    |
| CRM-B    | 87.01 ±0.40         | 21.11 ±0.06    | 65.91 ±0.34         | 1:3.12                    |
| CRM-C    | 88.16 ±0.04         | 21.45 ±0.00    | 66.71 ±0.04         | 1:3.11                    |
| CRM-D    | 88.34 ±0.12         | 21.61 ±0.24    | 66.72 ±0.12         | 1:3.09                    |
| CRM-E    | 88.68 ±0.12         | 24.41 ±0.12    | 64.27 ±0.33         | 1:2.63                    |

### Table 2. Total sugar and reducing sugar content of cassava root meal

| Sample   | Total Sugar (% DM) | Reducing Sugar (% DM) | Degree of Hydrolysis (%) |
|----------|--------------------|-----------------------|--------------------------|
| Cassava  | 3.28               | 1.04                  | 31.77                    |
| CRM-A    | 3.31               | 1.81                  | 54.52                    |
| CRM-B    | 3.28               | 2.06                  | 62.88                    |
| CRM-C    | 3.44               | 2.31                  | 67.20                    |
| CRM-D    | 3.83               | 2.59                  | 67.71                    |
| CRM-E    | 4.20               | 3.07                  | 72.99                    |

### 4. Conclusion

The different levels of isoamylase addition will produce cassava root meal starch with different chemical characteristics. The higher level of isoamylase addition can increase the total starch, amylose, total sugar, and reducing sugar content, and tend to reduce the amylopectin content as well as the amylose amylopectin ratio of hydrolyzed cassava root meal. The addition 0.05% isoamylase is able to make amylose/amylopectin ratio of hydrolyzed cassava root meal approached the corn.

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