# Proceeding

## The 2nd International Seminar

## Feed Safety for Healthy Food

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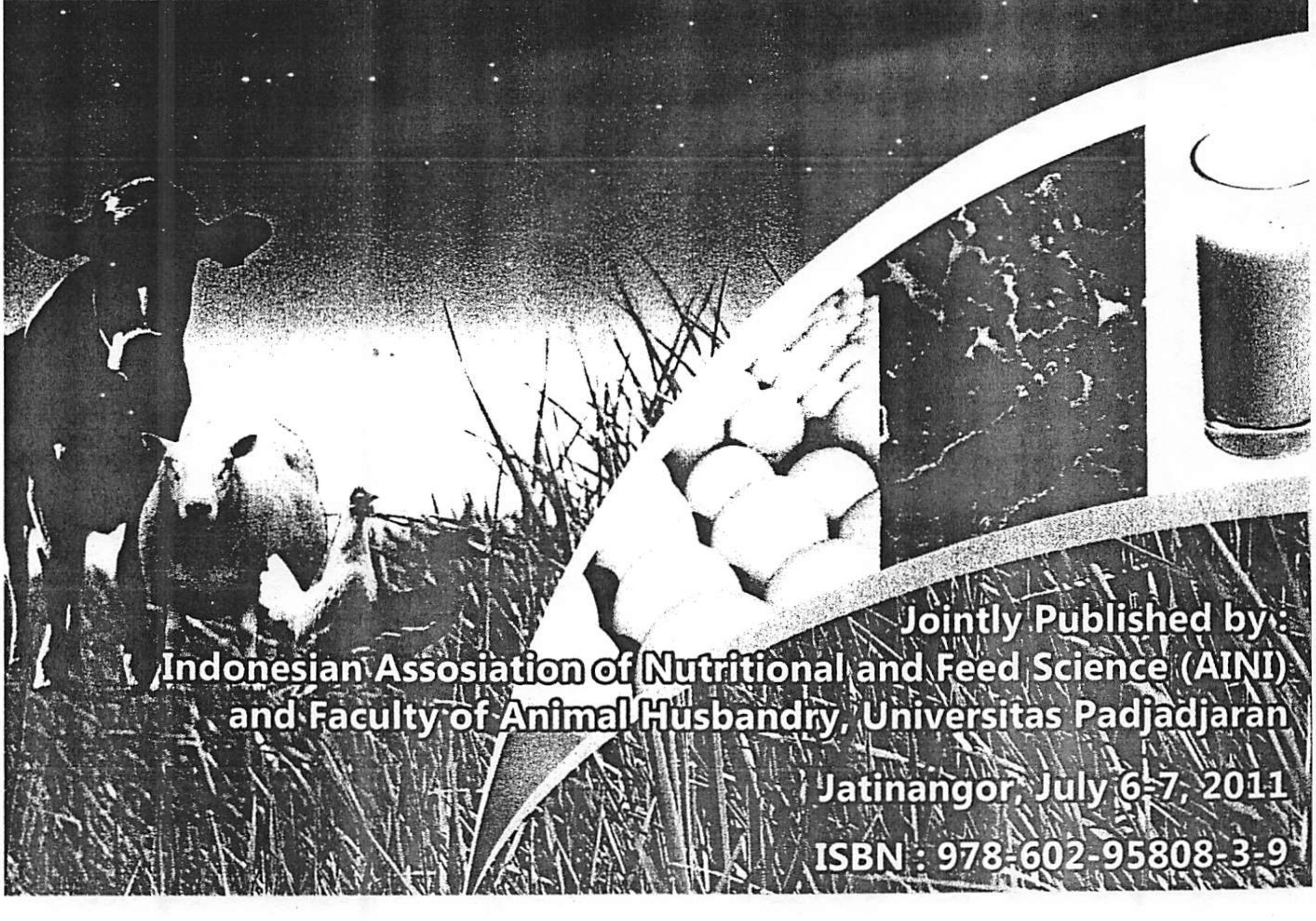

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## The 2nd International Seminar

## "Feed Safety for Healty Food"

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Technical Editors :
Secretariat of The International Seminar
"Feed Safety for Healty Food"

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The 2nd International Seminar
"Feed Safety for Healty Food"

**Keynote Speaker :**

Director General of Animal Husbandry and Animal Health

**Main Speakers :**

Prof. Fr. Jurgen Zentek (Berlin, German)
Prof. Abdul Razak Alimon (Malaysia)
Dr. Kevin Liu (Singapore)
Prof. E. R. Ørskov, Ph D., FPAS, FRSE (Scotland)

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The 2nd International Seminar
"Feed Safety for Healty Food"

**Subject Editors :**

Prof. Dr. Ali Agus

Dr. Ir. Kurnia A. Kamil, M. Agr.Sc., M. Phil.

Prof. Dr. Abdur Razak Alimon

Prof. E. R. Ørskov, Ph D., FPAS, FRSE.

Prof. Fr. Jurgen Zentek

Dr. U. Hidayat Tanuwiria, M.Si.

## FOREWORD

We thank the Almighty Allah, the Most Gracious and the Most Merciful that the proceedings of the 2nd International Seminar, the 8th Biannual Meeting and 3rd Congress and Workshop of AINI with the theme "Feed Safety for Healthy Food" organized by Indonesian Association of Nutrition and Feed Science, Faculty of Animal Husbandry, Universitas Padjadjaran on 6 - 7 July 2011 have been completed.

These activities were to collect variety of scientific information with the purpose to collect scientific information about feed for a healthy food, to produce a draft policy on a national feed system and to make a scientific forum for Academics, Researchers, Practitioners of animal husbandry, Health and Policy makers. Scientific papers that were presented either in oral or poster stated in the proceedings.

Thanks go to all those who have provided both moral support or material so that this seminar can be carried out and the proceeding can be issued.

Jatinangor, 5 March 2012

**Committee**

## CONTENTS

# THE EVALUATION OF FERMENTATIVE CAPABILITY OF CELLULOTIC FUNGI FROM COW RUMEN FLUID AGAINST DECREASE IN CRUDE FIBER AND READY AVAILABLE CARBOHYDRATE IN CASSAVE PEEL WASTE

**Yuli Andriani *, Ratu Safitri **,Abun*****

**) Faculty of Fisheries and Marine Sciences, Padjadjaran University, **)Biological Department of the Faculty of Mathematics and Natural Sciences Padjadjaran University, ***)Faculty of Animal Husbandry Padjadjaran University,

## ABSTRACT

This research had the purpose of finding out the decrease in crude fiber content in cassava peel waste (*Manihot esculenta* Crantz) through the fermentation proses with cellulotic fungi *Aspergillus tamarii* and *Penicillium nalgiovense* which were isolated from cow rumen fluid, and the efficienty of fermentation of the utilization the microbe in question. The product of fermentation will be use as a feed alternative for herbivore fishes. The method used was the descriptive and experimental method. The descriptive method was used to measure the sugar reduction content, *Dextrose equivalent* (DE), and to measure fungi growth (TPC) at the time of fermentation. The experimental method was used to find out the capability of cellulotic fungi isolate in question with respect to decline in crude fiber content. The research was conducted using the Completely Randomized Design (CRD) of the 3x3 factorial pattern with 3 replicates. Factor I is the fungi type, which consists of *Aspergillus tamarii*, *Penicillium nalgiovense*, consortium *Aspergillus tamarii* and *Penicillium nalgiovense*. Factor II is the inoculum dosage which consists of 1%, 2,5% and 5%.

The results of fermentation showed that *Aspergillus tamarii* at 1% was effective in lowering the crude fiber content dan had the best fermentation efficiency. The crude fiber content of cassava peel waste declined with a 20.85% decline percentage. The cassava peel waste fermentation (DE) efficiency utilized *A. tamarii* at 1% dosage, reaching 29.05% with an amount of sugar reducing agent of 15931.0345 mg/L. The occurrence of degradation of crude fiber was further reflected in celluosa and hemicellulosa composition after fermentation, where the cellulosa content declined 37.95% dan hemicellulosa as readily availabe carbohydrate increased 21%.

Keywords: cassava peel, fermentation, cellulotic fungi, cow rumen fluid, *Penicillium nalgiovense*, *Aspergillus tamarii*, crude fiber, fermentation efficiency (DE)

## INTRODUCTION

The approach to the problem of obtaining feedstuff which is cheap but meets nutrition requirements for fish is by maximalizing the use of cheap feedstuff, that is, carbohydrate, so as to possess better biological values, suppressing the use of protein as energy source in fish feed. One alternative of providing cheap fish feed raw material is by utilizing cassava peel waste as carbohydrate source, by enhancing its biological value through bioconversion process using cellulotic/amilolitic microbes from cow rumen fluid (Suharto, 1992).

The cassava planting area in West Java is about 113,663 hectares, with a total production yield of 2,044,673 tonnes annually, whereas the wasted peel is about 20% from the total wet based weight (BPS, 2009). The utilization of cassava peel as feedstuff faces the constraint found in agricultural waste in general, that is, a low protein value ((3.08-4.63%) and carbohydrate inside is present in crude fiber and its components, like lignin, cellulose, and hemicellulose which forms a complex bond so as to make it difficult for fish to digest. The constraints of fish feed to utilized carbohydrate in crude fiber is related to the availability of cellulotic enzymes which are limited in the fish digestive tract. This capability is also affected by the food habit of the fish, in which herbivore fish have the highest crude fiber digestive capability compared to carnivores and omnivores (Zonneveld, 1991). Several research reports stated that in the fish digestive tract, cellulase activity was found in small amounts (Saha and Ray 1998 ; Prejs and Blaszczyk 2006; Donovan et al 2004; Li et al 2004; Bairagi et al 2004; Nibedita et al 2008).

The process of simplying the carbohydrate complex bond may be carried out, among others, by using cellulotic/amilolytic microorganisms isolated from natural sources, like cow rumen fluid. Andriani (2010) has conducted isolation of cellulotic/amilolytic microorganisms in cow rumen fluid which can be utilized to degrade crude fiber in feedstuff. Research results indicated that there were 8 (eight) species of fungi which had cellulotic/amilolytic properties. Based on cellulotic index, the fungus isolate were chosen as cellulotic microbe candidate which was thought to possess the capability of highest degradation of crude fiber.

The potential of fungus cellulotic from rumen fluid in increasing carbohydrate digestive efficiency has not been optimally explored. Further research is needed which is expected to be capable of specifically conveying information on the fungus capability in decreasing crude fiber followed also by change in proportion in carbohydrate derivatives in crude fiber, that is, cellulose and hemicellulose which can be used as indicator of the availability of readily available carbohydrate in feedstuff. Further, this research was also meant to find out the fermentation efficiency (DE) of *Aspergillus tamarii* and *Penicilium nalgiovenses* cellulotic fungus in cassava peel waste substrate.

## MATERIALS AND METHOD

This research was conducted in the Microbiology Laboratory of the Biology Department of the Faculty of Mathematics and Natural Sciences, Padjadjaran University. The Design used was the Completely Randomized Design (CRD) with the 3 x 3 factorial pattern with 3 (three) replicates.

Factor I is the inoculum dosage (A) at level:

$a_1$ : 1 %

$a_2$ : 2,5 %

$a_3$ : 5 %

Factor II is the inoculum dosage (A) at level:

$b_1$ : *Aspergillus tamarii*
$b_2$ : *Penicillium nalgiovense*
$b_3$ : *Aspergillus tamarii+ Penicillium nalgiovense*

The parameters observed at the end of fermentation comprised crude fiber content, cellulose and hemicellulose, reducing sugar content and Dextrose Equivalent (DE). The data obtained were statistically tested by the ANAVA (Analysis of Variance), and in case of discrepancy, the data were further tested by the Duncan's multiple range test at 5% significant level (Gomez and Gomez, 2005).

## RESULTS AND DISCUSSION

### The Effect of Fungus Type and Inoculum Dosage On Decrease of Crude Fiber Content and Cellulose and Hemicellulose Proportion

The crude fiber content of cassava peel powder declined after seven-day fermentation. The highest decline was found by *A. tamarii* at 5% inoculum dosage, with a decline percentage of 28.83%, from 13.04% to become 9.28%.

Table 1. Percentage Decrease of Crude Fiber Content (%)

| Treatments | Crude Fiber (%) | Percentage of Decrease(%) |
|---|---|---|
| With no treatment | 13,04 | - |
| *Aspergillus tamarii 1%* | 10,32 | 20,86 |
| *Aspergillus tamarii 2,5%* | 9,80 | 24,85 |
| *Aspergillus tamarii 5%* | 9,28 | 28,83 |
| *Penicillium nalgiovense 1%* | 10,82 | 17,02 |
| *Penicillium nalgiovense 2,5%* | 10,06 | 22,85 |
| *Penicillium nalgiovense 5%* | 9,83 | 24,62 |
| *Aspergillus tamarii* dan *Penicillium nalgiovense 1%* | 10,61 | 18,63 |
| *Aspergillus tamarii* dan *Penicillium nalgiovense 2,5%* | 10,82 | 17,02 |
| *Aspergillus tamarii* dan *Penicillium nalgiovense 5%* | 10,62 | 18,56 |

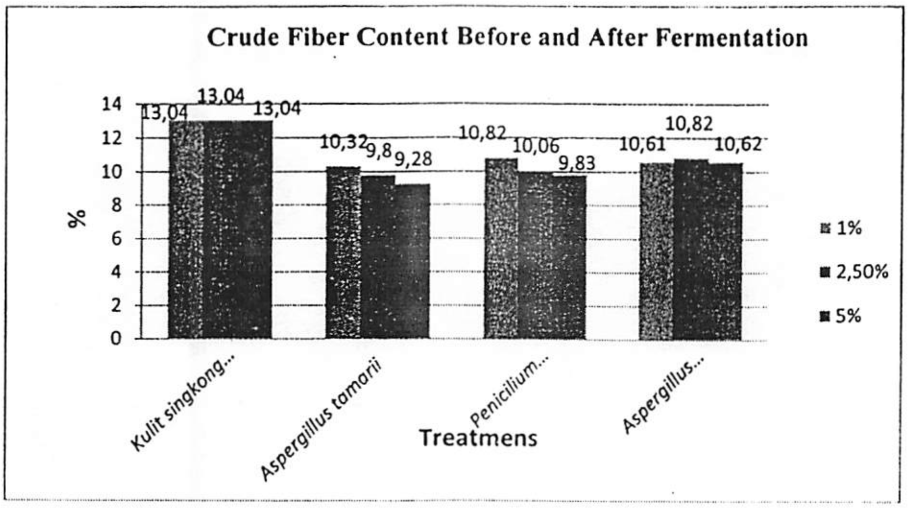


Fig.1. The Effect of Fungi Type and Dosage on Crude Fiber Content Before and After Fermentation

The result of the Anova test which was followed by the Duncan's Multiple Range Test ($p<0.05$) (Table 2) indicated that fungus type and inoculum dosage affected crude fiber content in the product as fermentation result of cassava peel waste.

Table 2. Duncan Test of the Effect of Inoculum Dosage (A) Interaction and Type of Fungus (B) on Crude Fiber Content of Cassava Peel as a Result of Fermentation.

| Dosage of inoculum (A) | Type of Fungus (B) | | |
|---|---|---|---|
| | *Aspergillus tamarii* | *Penicillium nalgiovense* | *Aspergillus tamari* dan *Penicillium nalgiovense* |
| 1% | 10,32 C<br>a | 10,82 B<br>b | 10,61 A<br>ab |
| 2,5% | 9,80 B<br>a | 10,06 A<br>a | 10,82 A<br>b |
| 5% | 9,28 A<br>a | 9,83 A<br>B | 10,62 A<br>c |

Note: Comparison of average values followed by the same letter is not significantly different according to the Duncan Test at a level of significance of 5%/ Lower case letters are read horizontally, Upper case letters are read vertically.

Based on crude fiber content values after fermentation (Fig 1), the fungus which effectively decreased crude fiber content was *A. tamarii* with a 5% inoculum dosage. This proved that *A. tamarii* possessed the capability of yielding cellulase enzymes capable of degrading cellulase. This statement conforms to Ward, et al. (2006), namely, that the *Aspergillus* sp genus produced several enzymes, including a-amylase, glucoamylase, cellulase, pectinase, xylanase and hemicellulase and protease.

The four essential classes of enzymes usually utilized in cellulase biodegradation, namely, endoglucanase, cellobiose, exoglucanases, and b-glucosidase, have been found in species of the Aspergillus. That is why the Aspergillus species is the natural "factory" for the production of enzymes like cellulase, xilanase, amylase, protease, and lipase. (Singh *et al.*, 1990 in Ward *et al*,2006).

The *A. tamarii* cellulase activity is higher than that of *P. nalgiovense*. This can be seen on the growth curve (Fig. 2). The growth curve shows that the growth of *P. nalgiovense* is higher than that of *A. tamarii*. This proves that with little growth *A. tamarii* is capable of lowering crude fiber content more compared to crude fiber decline by *P. nalgiovense*.

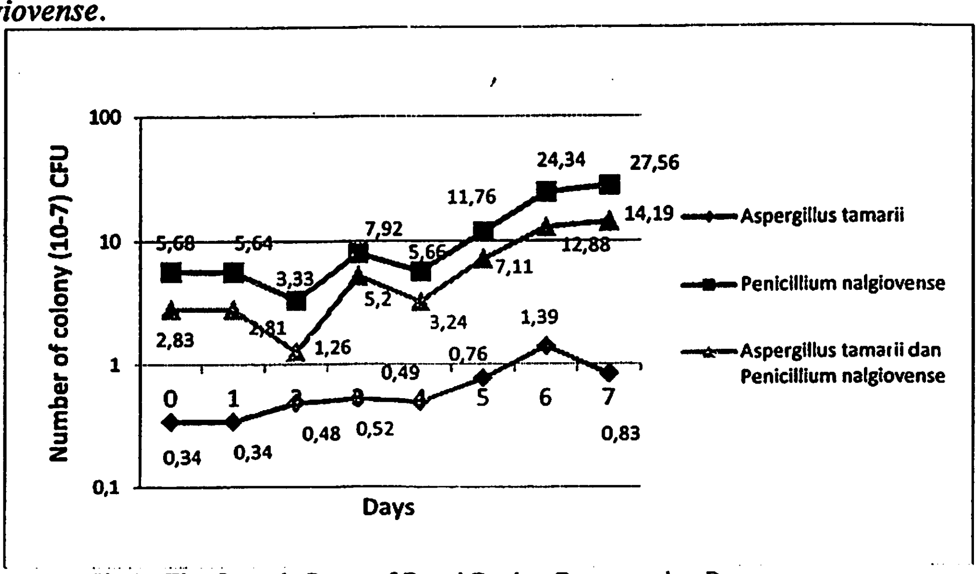


Fig.2. The Growth Curve of Fungi During Fermentation Process

Decline of crude fiber also occurred at treatment of *P. nalgiovens* fungi. This is due to the fact that *P. nalgiovense* can also produce cellulase (Nugraha, 2006). *P. nalgiovense* is capable of lowering crude fiber content to become 9.83 at 5% dosage. Lowering crude fiber content is in accordance with inoculum dosage, that is, the greater at fermentation process, *A. tamarii* as well as *P. nalgiovense*, the more the decline in crude fiber content.

The above is in accordance with the research conducted by Lusiana (2005) in Arief (2008), that is, that a low microbe dose still leaves a sufficiently high crude fiber content. An increasing dose of inoculum resuls in higher microorganisma activity.

Increase in inoculum dose in the fermentation process in the way of consortium is not in line with the magnitude of crude fiber decline. This is shown by decline in crude fiber at 1% and 5% dosage, which has crude fiber content value after fermentation which is almost the same. The 1% dosage is therefore deemed effective to lower crude fiber in fermentation by way of consortium.

The *A. tamarii* and *P. nalgiovense* consortium had a lower result of crude fiber decline. This was due to the fact of a suspected presence of competition between *A. tamarii* and *P. nalgiovense* fungus. The A. tamarii straing produced mycotoxin CPA/cyclopriozonic acid, fumigaclavine A, and kojic acid (Goto, et al., 1996), whereas the Penicillium genus is usually capable of producing mycotoxin and antibiotic β-lactam (Farber and Geisen, 1994). In spite of the fact, the two fungi mentioned can lower crude fiber content, but utilization by way of consortium is not recommended in lowering crude fiber content.

Based on the decline in crude fiber content, the *Aspergillus tamarii* and *Penicillum nalgiovensi* fungi from cow rumen fluid had the greater capability of lowering crude fiber content in cassava peel than that of the research of Rizal, et al., (2005). Rizal et al., (2005) indicated that cassava leaf fermentation by the *Aspergillus* *niger* fungi at 70% inoculum dosage lowered crude fiber content in the amount of 10.35%, whereas the decline in crude fiber content in this research was greater (28.83%) by using inoculum in smaller amount (1%).

Degradation of crude fiber content by the *A. tamarii* and *P. nalgiovense* fungi further affected cellulosa and hemicellulosa composition in cassava peel after being fermented (Table 3).

Table 3. Composition of Celullosa and Hemicelullosa Before and After Fermentation

| Treatment | Celullosa (%) | Hemicelullosa (%) |
|---|---|---|
| Without fermentation | 6,06 | 2,48 |
| *Aspergillus tamarii 1%* | 3,76 | 3,0 |
| *Aspergillus tamarii 2,5%* | 3,45 | 2,48 |
| *Aspergillus tamarii 5%* | 4,73 | 1,73 |
| *Penicillium nalgiovense 1%* | 3,35 | 1,72 |
| *Penicillium nalgiovense 2,5%* | 3,74 | 1,59 |
| *Penicillium nalgiovense 5%* | 3,89 | 1,58 |
| *Aspergillus tamarii* dan *Penicillium nalgiovense 1%* | 3,88 | 2,99 |
| *Aspergillus tamarii* dan *Penicillium nalgiovense 2,5%* | 3,90 | 2,37 |
| *Aspergillus tamarii* dan *Penicillium nalgiovense 5%* | 3,36 | 2,37 |

Fermentation using cellolitic fungus from cow rumen fluid lowers cellulosa and hemicelullosa as crude fiber components in cassava peel. Further, there was a change in hemicellulose in fermentation product, which was an indicator of readily available carbohydrate availability, which was the highest in treatment using *A. tamarii* 1% (3.00%).

**Fermentation Efficiency Through *A. tamarii* and *P. nalgiovense* Fungi of Cassava Peel Substrate**

Dextrose Equivalent (DE) is the percentage of the amount of starch polymer cut to become simple sugar (glucosa), in other words, DE is the percentage of sugar overhaul from the total starch content. The value of DE was obtained from the proportion of reducing sugar concentrate to the total sugar concentrate; the value of DE increases in line with the increase of reducing sugar.

Reducing sugar is a group of sugars (carbohydrate) capable of reducing electron acceptor compounds, for example, glucose and fructiose. The end of a reducing sugar is the end that contains aldehyde or free ketone groups. All monosaccharides and disaccharides belong to reducing sugars. Generally the reducing sugar produced is closely related to enzyme activity. The amount of reducing sugar during reaction was measured using the reactant dinitro salicylicacid at a wavelength of 540 nm. The higher the value of absorbance produced, the greater the content of reducing sugar. (Anonim[2],2011)

Fungi activity causes an overhaul of cellulose as well as hemicellulose and lignin becomes glucose (simple sugar) which can be utilized by the fungi to grow and breek (Ningrum, 2008). The decrease in crude fiber content will be followed by an increase in reducing sugar content, because glucose is a product of the result of degradation of crude fiber itself. Fardiaz (1988) stated that microbes will use carbohydrate as energy source by way of breaking it to become simple sugar like glucose (Anwar, et al., 2009).

The result of reducing sugar analysis showed that the type of fungus which produced the highest reducing sugar content at the end of fermentation was *A. tamarii* 1%. This is probably due to the fact that *A. tamarii* fungi has high cellulase enzyme activity.

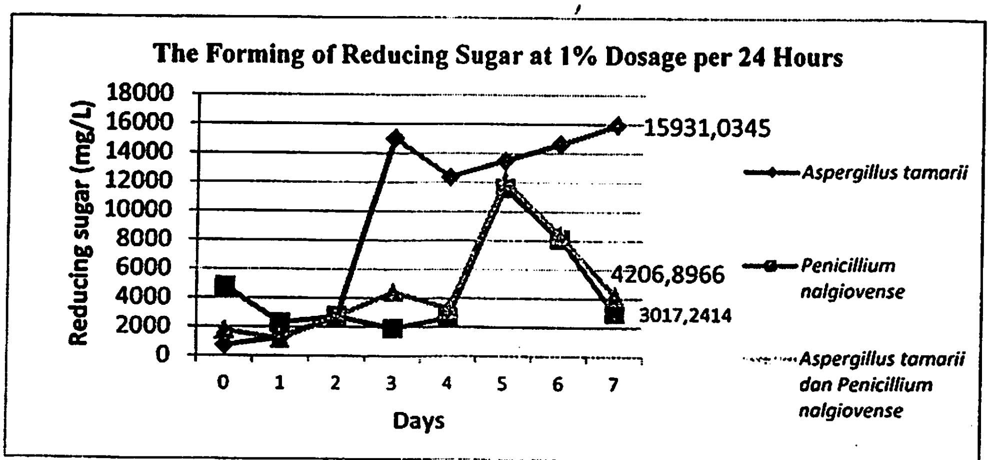


*Fig.2. The Forming of Reducing Sugar at 1% Dosage per 24 Hours*

*By the above figure, one can find that the highest reducing sugar content at the end of fermentation is produced by A. tamarii 1%. This indicates that A. tamarii has the best hydrolysis capability compared to other fungi with an average highest sugar reducing at the end of fermentation, that is, 15931.0345 mg/l. The change in sugar reducing content is caused by the fact that the fungus carries out hydrolysis activity. The hydrolytic process consists of two stages, namely, cellulose degradation to become cellobiose because of endo-β-1,4-glucanase and exo-β-1,4 continues to the breaking of cellobiose because of β-1,4 glucosidase (Anwar dkk.,2009).*

$$selulosa \xrightarrow[\text{ekso}-\beta-1{,}4\ \text{glukanase}]{\text{endo}-\beta-1{,}4-\text{glukanase} +} selobiosa \xrightarrow{\beta-1{,}4\ \text{glukosidase}} glukosa$$

*The substrate was hydrolysed to become monosaccharide, disaccharide, or oligosaccharide as source of carbon for cell growth and carry out metabolim also as a means to obtain energy (Fardiaz, 1988). This can explain the fluctuating pattern of change in sugar content in Fig. 2.*

Increase in sugar content came about due to hydrolysis of cellulose to become simple sugar. The conversion of starch into simpler sugar was caused by extra-cellular enzymes secreted by the growing fungus during the fermentation process. The decrease in reducing sugar content, on the other hand, was due to the fact that the fungi used the sugar produced again for its growth. After saccharification, the sugars formed would be used by microbe growth (Gunadnya and Antara, 1997). The above is in accordance with what was stated by Hays (2004), that almost all glucose products produced from the overhaul of cellulose and hemicellulose was used by microbes in ruminant cow rumen for cell growth and division.

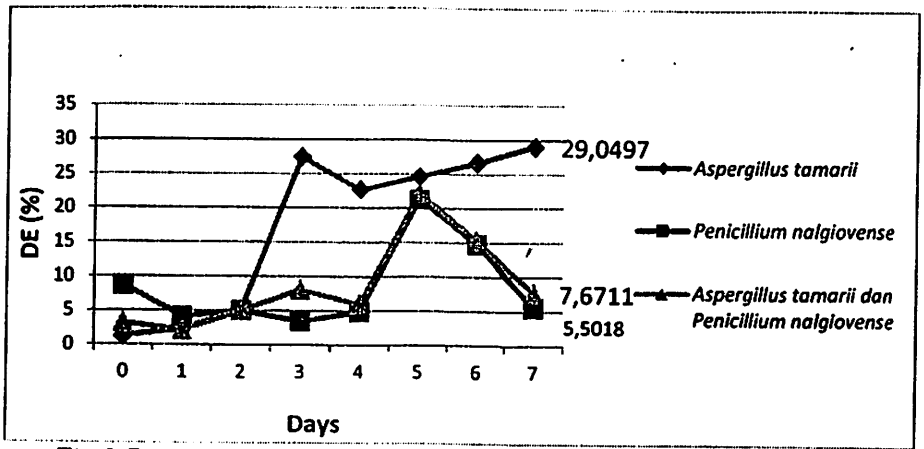


*Fig.3.Dextrose Equivalent (DE) at 1% Dosage per 24 Hours*

Fig. 3 shows that the highest DE value at the end of fermentation is 29.05% by *A. tamarii* fungus at 1% dosage. The reducing sugar content in the fermentation process experienced increase and decrease, so that the DE experience increase or decrease in line with the reducing sugar content. The higher the reducing sugar content the greater the DE value.

The DE value obtained from all fermentation treatments by the *A. tamarii, P. nalgiovense* fungi and the *A.tamarii* and *P. nalgiovense* consortium did not reach the 100% value. This was probably due to the fact that the cellulase enzyme activity produced by the fungus was still sufficiently low. The fungi used (*A. tamarii* and *P. nalgiovense*) were not pure cellulotic, making fiber degradation capability still low. This is also the case with *P. nalgiovense* which is also proteolytic in nature and which could produce protease enzymes (Farber and Geisen, 1994).

## CONCLUSION

The fermentation results showed that *Aspergillus tamarii* at 1% dosage was effective in lowering the crude fibre content and had the best fermentation efficiency. The crude fibre content of cassava skin effluent decreased with a percentage decrease of 20.85%. Fermentation efficienty (DE) of cassava skin effluent utilized *A. tamarii* at 1% dosage reached 29.05% with the amount of sugar reducing agent of 15931,0345 mg/L. Degradation of crude fiber was further reflected in cellulosa and hemicellulosa composition after fermentation, where the cellulosa content decreased 37.95% and hemicellulosa as readily available carbohydrate component increased 21%.

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