EFFECT OF DOSAGE *Rhizopusoligosporus, Aspergillusoryzae, Trichodermaviride,* and *Trichodermareesei* ON VIABILITY, Reducing sugars, AND NUTRITIONAL VALUE OF CORN COBSs FERMENTATION

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ABSTRACT

A research toward the effect of single culture of Rhizopusoligosporus, Aspergillusoryzae, Trichodermaviride, and Trichodermareesei with various dosages of inoculums and incubation periode toward viability, sugar content of reductant and improvement of nutrition quality of corncob has been done. The purpose of the research was to find out about the extent of viability of the fungi on corncob substrate, and the capability in decomposing the substrate to improve the nutrition quality. The descriptive method was used for nutrition quality analysis (crude protein, crude fiber and crude fat), the complete randomized block design was used for the measured of fungi growth (the amount of fungi (TPC), pH, temperature and water content), and the sugar content of reductant for 8 days of fermentation process. The research result showed that the fermentation process was carried satisfactorily, while the reductant sugar content produced was also increasing. The analysis result on nutrition content showed that 6 days fermentation was the optimum time for fungi growth. Trichodermaviride with dosage 3% and incubation periode in 6 days is the best fungi that can growth in corncobs. The highest rate of reductant sugar produced by Trichodermaviride with dosage 3% and incubation periode in 6 days. The fungus that would increase the nutrition content of corncob was Rhizopusoligosporus with dosage 3% and incubation periode in 6 days the highest crude protein 8.95%, the lowest crude fiber 19.49% and crude fat 1.85%.

Keywords: *Rhizopus oligosporus, Aspergillus oryzae, Trichoderma viride, Trichoderma reesei, inoculum dosage, incubation periode, corncob, crude protein, crude fiber, crude fat, fermentation.*

PREFACE

1.1.1. Back Ground.

Feed represent one of determinant success of an effort of breeding and fishery. Supply of cheap feeding stuff, good quality and do not have the character of poison need to be performed to depress production cost, because presently 60 % from production cost component is feed supply cost (Mirwandhono, 2004). One of the effort

performed to drop cost is by utilizing source of feeding stuff which has low economic value, do not vie with human, and available continuously. Source of the feeding stuff obtainable by the way of utilizing agriculture waste, or plantation waste which still be not common used (Sinurat,dkk., 1999).

One of agriculture waste which only a little utilized is waste from agriculture of corn. Corn production in Indonesia reachs 11.737.000 tons or increases 4,56% compared to year before. Number of the production produces agriculture waste, one of them are cob which is 30% from weight of corn, therefore in its 1 years obtained by around 2 million cob tons per year (Agricultural Department, 2004). Cob has not been utilized in an optimal fashion, and usualy only thrown or burned, seldom used as component of foodder. Exploiting of cob as component of feed, limited by content of low nutrient, and height of crude fiber rate so that difficult digested by livestock.

The problems can be overcome through bioprocess cob becomes feeding stuff. Fermentation represents a processing bioprocess technology of by entangling activity of a microorganism in a hope that can improve quality of food-stuff nutrient which with quality low. Fermentation gives result which more beneficial compared to processing in physical and chemical, because do not endanger, do not generate pollution and do not produce poison (Doyle,1986). Fermentation there performed through the enzyme secretion by microorganism so that produces digestible substrate and can enrich substrate with microbe protein (Doyle,1986).

Used microorganism must stay in active state so that takes a short cut adaptation time, be available in qs so that can produce inoculum in optimum measuring, and free of contaminant (Rahman, 1989). Some of the fungi which can be utilized for material fermentation which comes from agriculture waste of among others Rhizopusoligosporus, Aspergillusoryzae, Trichodermaviride, and Trichodermareesei, because are able to use cellulose as source of carbon to live, and are able to improve;repair feeding stuff which its nutrition low. Fungi monoculture used to know fungi type which gives the best result in improvement of quality of cob nutrient, because every fungi type has ability of different life though at the same substrate (Mirwandhono, 2004).

Success of a fermentation process in producing product, better and with quality higher compared to material from it, closely related to way of processing. Inoculum dose or microorganism type which is used able to affect expected end product. Dose level relates to microorganism population. This thing sets the pace development of microorganism to radically change substrate. Inoculum dose excelsior, more and more microorganism population. This thing causes substrate component which dirombak increases (Darwis, dkk.,1990). Inoculum usage proportion usualy between 3-10% from media total volume but in big scale earns also uses 1-3% inoculum volume (Judoamidjojo 1989, Wibowo, 1990).

1.2. Purpose of Research

This research intention is know fungi type influence, inoculum dose, and time of fermentation to improvement of quality of knowable cob nutrient passes growth of fungi, change of degree of acidity (pH), temperature, water content, and reducing sugar rate during fermentation process. Intention of this research is get one of fungi type, inoculum dose, and time of fermentation which gives result of optimal fermentation.

MATTERIAL AND METHOD

2.1. Equipment and Material

Equipment which is used in this research, for example: beater, stir bar, blender, brown bottle, medium bottle, alumunium cup, solder cup, porcelain cup, funnel shaped buchner, funnel shaped of buffer, eksikator, erlenmeyer, chemical glass, graduated cylinder, hot plate, incubator, jara, plastic sack;bag, cotton, marble, newsprint paper, filter paper, liquefier, cuvette, lable, boil gourd, gourd Kjeldhal, lamp, fermentor cupboard; locker, autoclave, ose, oven, pan, bunsen heater, water bath, PH meter, spotting pipette, volume pipette 1 mls, volume pipette 10 mls, test tube rack, soxhlet, spectrophotometer, test tube cork, test tube, electric furnace, weighing-machine, and thermometer.

Material which is used in this research, for example: alcohol 70%, alcohol 80%, jelly bar, akuades, sulphate acid 1,25%, acetone, rice, phosphate buffer, chloramfenikol, glucose, mixed indicator (bromic cresolgreen and red methyl) isolate

Rhizopusoligosporus, *Aspergillusoryzae*, *Trichodermaviride* and *Trichodermareesei*, mixture catalyst (CuSO4.5H2O and K2Hso4), chloroform, physiological NaCl condensation, molasses 5%, NaOH 1,25%, NaOH 40%, copper sulphate reactant, reactant Arsenomolibdat, Potato Dextrose Agar (PDA), spirtus, and cob flour.

2.2. **Methode**

Method which is used in this research is descriptive method and eksperimental. Descriptive method used at result of the proximate analysis consists of crude protein rate, crude fiber, and crude fat, while method eksperimental used to analyse number of fungi (Total Plate Count), change of hydrogen ion exponent, temperature, water content, and reducing sugar rate by using Completely randomized design (RAL) factorial pattern 4 x 3 x 8 with 3 times repetition.

Factor I is fungi type monoculture (A) with four levels are :

a1: Cob flour which is inoculation *Rhizopusoligosporus*

a2: Cob flour which is inoculation *Aspergillusoryzae*

a3: Cob flour which is inoculation *Trichodermaviride*

a4: Cob flour which is inoculation *Trichodermareesei*

Factor II is inoculum dose (B) with level :

b1: Inoculum dose 1 %

b2: Inoculum dose 2 %

b3: Inoculum dose 3 %

Factor III is fermentation time (C) with level :

c1: old of fermentation 1 days

c2: old of fermentation 2 days

c3: old of fermentation 3 days

c4: old of fermentation 4 days

c5: old of fermentation 5 days

c6: old of fermentation 6 days

c7: old of fermentation 7 days

c8: old of fermentation 8 days

2.3. Parameter

Parameter which is measured at research among others content of nutrient like crude protein, which is performed by every 2 days. Besides, measured by full scale of fungi (Total Plate Count), and reducing sugar rate, and which is performed by every twenty-four hours.

2.3.1. Total Plate Count (TPC)

Full scale counting of fungi there performed according to Total method Plate Count (TPC) (Cappucino and Shjerman, 1987). Samples there deliberated 1 grams, then there packed into 10 mL physiological Nacl 0,9 %. Then, shaked to homogeneous, so that gotten first thinning (10^{-1}).

From first thinning taken by 1 mL then there packed into test tube contains 9 mL physiological Nacl got second thinning (10^{-2}). Then performed by the same step up to performed thinning

($10^{-(y-2).}$).

Three final thinning (10^{-y},$10^{-(y-1)}$,$10^{-(y-2)}$) , individual taken by 1 mL and packed into by different steril solder cup, then into the solder cup added by Chloramfenikol 0,5 magnesiums and poured by medium Potato Dekstrose Agar (PDA)) steril which have been made cool till 400C and. Then, homogenized with by doing to move to turn around to propagate microbe cells. Incubation there performed during 2 days.

Fungi colony which grows at each solder cup there counted him(her. If the numbers had not countable or too a few, then are not packed into calculation formula.valid or certifiable Value spread is 30-300 colonies in 1 solders. After calculated, number which is gotten of every solder there packed into calculation formula.

Certifiable value is thinning $10^{-(y)}$ (a), $10^{-(y-1)}$ (b), $10^{-(y-2)}$ (c),then :

$$\text{Jumlah jamur} = \frac{(a \times 10^{y}) + (b \times 10^{y-1}) + (c \times 10^{y-2})}{3}$$

Result of calculation there got number of fungi per substrate gram (CFU / gram). Then made by the growth curve with axis X incubation time and coaxial Y number of fungi. Total Plate Count (TPC) performed by every twenty-four hours.

Determination Procedure of Crude Protein Rate (Method Kjeldahl)

a. Destruction

1 dry sample grams packed into by Kjeldahl flask, and added by 6 mixture catalyst grams ($CuSO_4.5H_2O$ dan K_2HSO_4 with comparison of 1:5) and added by 20 acids mLs of condensed sulphate.Then, heated by above small flame in sour space, if had not seen effervescence there used big fire. Destruksi there assumed completing if condensation have with clear green colour, then made cool by and ready for didestilation.

b. Destilation

Result condensation of destruksi removed from Kjeldahl flask into boil gourd, then there rinsed with akuades 50 mL. Erlenmeyer which have been filled sour of borax 5% 10 mL and have been given mixed indicator (bromic cresolgreen : red methyl = 4:5 0,9 grams, mixture dissolved in alcohol 100 mL) set at distilleries. Mixed indicator which is used by 2 drips. Result condensation of destruksi alkaline by adding 40-60 mL Naoh 40% through funnel beside, and closed of funnel shaped faucet. Bunsen heater there flamed and destilation there started till the condensation accomodated in erlenmeyer at least 15 mL.

c. Titration

Gourd erlenmeyer which contains titration supernatant with HCL 1 N. Point of titration have been reached with marked by condensation discoloration becomes pink. Countable crude protein rate with formula as follows:

$$\textbf{\% crude protein} = \frac{ml\ HCl\ x\ N\ HCl\ x\ 0.014\ x\ 6.25}{berat\ sampel\ (g)}\ \textbf{x 100\%}$$

RESULT AND DISCUSSION

3.1. Fungi Type Influence, DosisInokulum, and Tipe of Fermentation To Number of Fungi (CFU / g) At Cob Fermentation

Success of fermentation process is very determined by ability of microorganism to grow and with growth at substrate (Raimbault, 1998). During fermentation, performed counting of number of colony as a mean to know its growth at substrate.

Number of fungi increases during fermentation process there compared to inoculation beginning. Number of fungi when inoculation beginning that is 108 CFU / g. Growth of

fungi during cob fermentation, visible in the Enclosure 2. Result of analysis of variance in the Enclosure 3 indicating that treatment of fungi type, inoculum dose, and old of fermentation, and interaction between fungi type and inoculum dose, inoculum dose and old of fermentation, fungi type and old of fermentation and the three interaction influence has an effect on to growth of fungi (viability) during fermentation process.

The next, to know influence each treatment to growth of fungi (viability) during fermentation process, then performed Duncan's test.

Table 1. Fungi Type Interaction Influence (A) and Inoculum Dose (B) To Number of Fungi Colony (CFU / gr) Duncan Test

InoculumDose (B)	Fungi Type (A)			
	a1	a2	a3	a4
b1	11,46 a A	12,30 a A	12,38 a A	11,42 a A
b2	11,56 a AB	11,89 ab A	12,91 b A	11,63 a AB
b3	12,38 a B	12,77 a A	12,97 a A	12,36 a B

Description :

Comparison of plane value - plane which is followed by the same letter do not real different according to Duncan's test at significant level 1 %. Lower cases there read horizontal direction, letter kapital there read vertical direction. a 1= R..oligosporus, a2 = A. oryzae, a3 = T. Viride, a4 = T. Reesei b1 = dose 1%, b2 = dose 2%, b3 = dose 3%

At Tables 1 indicating that fungi *Trichodermaviride* with inoculum dose 3% has highest viability at cob fermentation. Addition of dose causes improvement of number of fungi, and speed of growth at each dose differs. Inoculum dose which excelsior will produce number of fungi yamg more and more (Tangendjaya, 1993). Based on result of Test Duncan to number of fungi with existence of fungi type interaction (A) and Inoculum Dose (B) show that fungi type *Rhizopusoligosporus, Aspergillusoryzae, Trichodermaviride, and Trichodermareesei* produces highest growth at dose 3%. But, at

Aspergillusoryzae dose 1% shows higher level growth compared to dose 2%, therefore for cob fermentation by using fungi dose 1% can produce fair growth.

Growth of each highest fungi at dose 3%. This thing in line with research of Muhiddin (2001) what expresses that used inoculum dose excelsior, then mycelium which is formed will more and more. This thing depend on ability of adaptation each fungi type to substrate. Growth of fungi with existence of interaction between visible fungi type and inoculum dose at Figure 1.

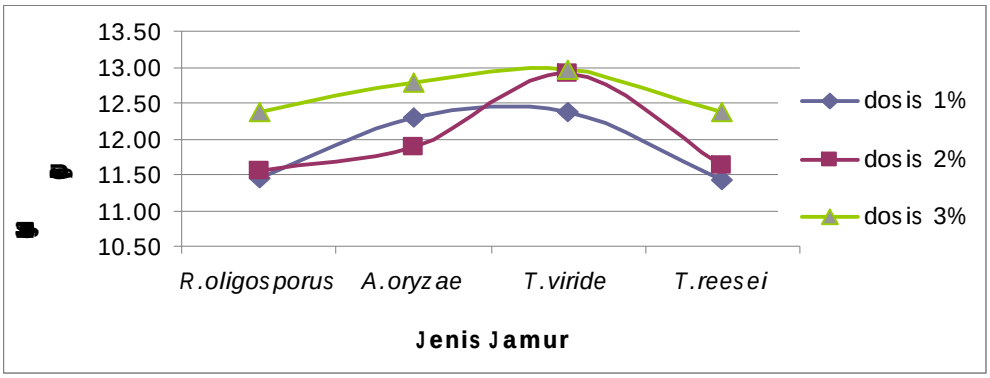

Figure 1.Logaritmic number of colony with existence of fungi type interaction and inoculum dose.

Average of growth of *Trichodermaviride* (a3) at dose 1 %,2 %, and 3 % higher compared to the other fungi type (Figure 1). This thing indicates that *Trichodermaviride* has ability of better adaptation at cob waste, while *Trichodermareesei* has ability grows and with kembangbiak at lower cob substrate, so knowable that *Trichodermareesei*less match used in cob fermentation there compared to other fungi.

3.2. Fungi Type Influence, Inoculum Dose , and Time of Fermentation to Change of Crude Protein content of Fermentation Cob.

Table 2. Crude Protein content (%) of Fermented Cob

Day	**Dose**	**Fungi Type**			
		Rhizopus oligosporus	***Aspergillus oryzae***	***Trichoderma viride***	***Trichoderma reesei***
2	1%	6.71	6.34	6.73	5.9
	2%	6.66	6.28	6.94	6.72

	3%	8.07	7.5	7.48	6.69
4	1%	8.18	7.53	7.26	6.03
	2%	7.44	7.52	7.04	6.92
	3%	8.03	7.99	8.35	6.81
6	1%	8.61	7.8	7.3	6.38
	2%	8.64	7.41	8.03	7.73
	3%	8.95	8.33	8.58	7.58
8	1%	7.4	7.33	6.26	6.08
	2%	7.02	6.28	7.04	6.14
	3%	7.89	6.91	7.36	6.74

Fermentation with *Rhizopusoligosporus* produces improvement of highest crude protein rate compared to the other fungi type. *Rhizopusoligosporus* is able to increase crude protein of cob from 2,57 % becomes 8,95 % at day ke-6, this thing in line with the research performed by Muhiddin (2001), that usage of fungi *Rhizopusoligosporus* at wood corm skin can increase crude protein content from 3.41 % becomes 8.3 % while *Aspergillusoryzae* is able to increase crude protein between becoming 6,28% at day second becoming 8,33% at day ke-6. *Trichodermaviride*produces increase of higher crude protein compared to *Aspergillusoryzae*, that is 8,58% at day ke-6. Result of this research in line with result of research of Abou-zeid (1991) what expresses that paddy bran fermentation with *Trichodermaviride* can increase paddy bran protein from 9,07% becomes 14,61%, corn bran protein content increases from 3,06% becomes 5,15%, while *Trichodermareesei* only be able to increase crude protein becomes 7,58%, or lowest compared to other fungi. Change of crude protein of cob which is fermentation by *Rhizopusoligosporus* which indicates that crude protein increases from day ke-0 to day ke-6, while at day ke-8 experiences degradation. Inoculum dose 3% produces improvement of highest crude protein compared to dose 1% and 2%.

Cob fermentation by *Aspergillusoryzae* show old of fermentation 6 days produces increase of highest protein with dose 3%, the same thing happened at

fermentation by *Trichodermaviride*, while at *Trichodermareesei* inoculum dose 2 % and 3 % do not show difference to crude protein content of substrate.

Difference of improvement of protein content caused by difference of ability of each fungi in radically changing substrate. Besides, affected also by the enzymes produced by every fungi type. Fermentation by proteolytic ufngi like *Rhizopusoligosporus* will increase protein condensation. This thing referring to the ability of proteolytic enzyme to decompose protein becomes amino acid, therefore nitrogen there dissolved experiencing improvement, (Suliantari and Rahayu, 1990). *Aspergillusoryzae* represents source of lipase enzyme and protease. Protease enzyme breaks protein becomes peptide and amino acid. During fermentation happened happened extract conversion becomes sugar (Winarno, 1983). improvement of protein, proven by that optimal improvement obtained at dose 3%. More and more high of inoculum dose, more and more fungi population, therefore more and more the mycelium formed along also with that thing will increase total nitrogen content proportionally, because degrading of crude fiber. This thing happened because for growth of fungi there required alteration of carbohydrate from substrate (Darana, 1995). But, in the end alteration of substrate by fungi determined by fungi population balance and substrate nutrient. Therefore, at improvement of dose 3 %, the result is not far is differ with dose 1 % and 2 %.

Improvement of the protein happened during fermentation runs resulted by caused by activity from fungi and caused by addition of protein which is rendered by fungi body as result of its growth (Tangendjaya, 1987). The same things presented by Abou-zeid (1991) that increase of substrate protein. Improvement of inoculum dose or time of fermentation have increased crude protein rate. Based on during fermentation indicates that fungi is able to use part of substrate for its growth and forms protein microbial. Sinurat (1999) arise that the increasing of food matters as result of fermentation there resulted from two possibilities. Firstly, improvement of caused by mycelium. Second, as result of the happening of change of other food matters, like degradation of crude fiber.

Based on the result obtained by and explanation of before, then can be told old treatment of fermentation gives opportunity at inoculum to go on using compiler

components of substrate for continuity of its living. The longer fermentation time, mean more and more give opportunity for inoculum to grow and with growth, therefore number of component which can be turned into cell mass also more and more.

During growth, fungi needs nitrogen for fungi cell protein synthesis, to fulfill its requirement with media protein hydrolysis and then experiences deamination. Inoculum concentration excelsior which is used for fermentation of a substrate, need shorter incubation time compared to which its concentrating lower to reach desired optimum product (Suwandyastuti, dkk.,1995).

CONCLUSSION

1. .Fungi type which has best viability at cob substrate is Trichodermaviride with inoculum dose 3% and old of fermentation 6 days that is 3.95x1014 CFU / g
2. Fungi type which produces highest reducing sugar rate is Trichodermaviride with inoculum dose 3% and old of fermentation 6 days that is 64839,4 mg/L.
3. Rhizopusoligosporus with inoculum dose 3% and old of fermentation 6 days gives the best influence at improvement of quality of cob nutrient that is crude protein 8,95%, crude fiber 19,49%, and crude fat 1,85%.