EFFECT OF MIXTURE INOCULUM OF LACTIC ACID BACTERIA (LAB) AND MOLD AMYLOLYTIC IN VARIOUS CONCENTRATION AND FERMENTATION TIME OF CHANGING PROTEIN AND HCN CONTENT OF BITTER CASSAVA ROOTS (*Manihot aipi* Phol.)

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Abstract. The purpouse of this study were to determine the effect of mixture inoculum of lactic acid bacteria (LAB) and mold amylolytic in various concentration and fermentation time of changing protein and HCN content of bitter cassava roots during fermentation of bitter cassava roots. Inoculum used is a mixture of LAB and molds amylolytic isolated from "Wikau Maombo" fermented with the highest amylolytic activity. This study was an experimental research in the pattern completely randomized design (CRD) two factorial. Substrate of bitter cassava roots inoculated as many 5%, 10% dan 15% of inoculum consentration and incubated at room temperature for 8th days. Method of measuring protein content used the Biuret method, whereas the method of measuring the HCN content used argentometry method. Analysis of data used SAS software (Statistical Analysis System). The result of this research showed that the highest of protein content was 177,28 mg/g, whereas the lowest HCN was 16,42 mg/kg on the 6th days of fermentation used 15% of inoculum consentration.

Keywords: LAB, Mold, Protein, HCN, Bitter Cassava Roots.

INTRODUCTION

Peoples in Southeast Sulawesi has long processing bitter cassava root through traditionally fermentation into "Wikau Maombo". Nutrient content in bitter cassava root which are proteins 0.7221%, 0.0639% fat, starch 70.16%, 0.61% reducing sugar and cyanide (HCN) 103.8352 ppm (Muhiddin *et al*., 2014) , High starch content in tubers of cassava allows for the growth of various types of microorganisms which have amylolytic capable.

Microorganisms which has high amylolytic capability, can be applied to the fresh starch fermentation of cassava roots. The success of traditional fermentation mainly lies in the number and types of microorganisms that play a role in the fermentation process. Inoculum concentration and types of microorganisms that play a very decisive role of fermentation product end, such as nutrient content, texture, flavor and aroma (McNeil and Harvey, 1990; Sahlin, 1999; Odoemelam, 2005). Fermentation of cassava roots can be made using natural microorganisms or pure cultures as well as with a single or culture a mix.

The microorganism which has the ability amylolytic potential to be used for fermentation with substrate raw cassava tubers. Lactic acid bacteria and Rhizopus which has the highest amylolytic ability of the results of the first study year (Muhiddin and Munir, 2014; Muhiddin and Munir, 2015) developed in mixed cultures become "ragi wikau maombo".

Lactic acid bacteria (LAB) are a group of Gram-positive bacteria, do not form spores, cocci or rod and produce lactic acid as the main end product of fermentation of carbohydrates. Based on the taxonomy of

lactic acid bacteria has several genera including Aerococcus, Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Oenococcus, Pediococcus, Streptococcus, Tetragenococcus, Vagococus, and Weissella (Salminen et al., 2004)

LAB contained in the fermented cassava starch as subrate utilizing known as LAB amylolytic. The activity of lactic acid bacteria in the fermentation of starchy materials contribute to changes in product characteristics to produce lactic acid and the enzyme amylase. LAB amylolytic instrumental produce antimicrobial compounds that inhibit pathogenic bacteria and spoilage bacteria that may contaminate food products of fermentation, and lower levels of HCN in the fermentation of bitter cassava tubers (Petrov et al., 2008; Muhidin, 2011)

Lactic acid bacteria produce lactic acid as the main product of the fermentation of carbohydrates (Steinkraus, 2002 and Williams and Dennis, 2011 in Oyedeji et al. (2013). Pratama *et al.* (2013) also states that the fermentation using Lactic Acid Bacteria (LAB) were able to remodel complex compounds into simpler compounds with the end result that is lactic acid. lactic acid can produce a low pH value on the substrate resulting in acidic conditions. the decline in the pH values can slow the growth of spoilage microorganisms on food.

Benefits fermentation using lactic acid bacteria (LAB) is on quality improvement (increase in aroma, flavor, and texture, and others). Improved digestibility, which leads kepengurangan preparation time and energy required. The formation of essential amino acids, essential fatty acids and vitamins and detoxification during fermentation. In addition, improving the quality of various food commodities such as cocoa and tape (Kostinek *et al*., 2008)

Results Muhiddin and Munir (2014) showed that the fermented cassava tubers using a mixture of BAL and mold inoculum amylolytic able to increase the protein content of cassava tubers from 5.90% to 13.41% and lower levels of HCN 13.20 into 11.60% during the four days of fermentation with a concentration ratio of 1: 1. However, the concentration of inoculum and optimum fermentation time is not yet known. Based on this, the need for further research on the effect of concentration and fermentation time BAL and mold inoculum mixture amylolytic to changes in protein and HCN cassava tubers fermented products.

MATERIAL AND METHOD

Materials

The mainly material used in this study are tubers of bitter cassava varieties obtained from Lantongau Village, District Central Mawasangka, Buton Central. Amylolytic LAB and molds isolates used are isolated from "Wikau Maombo" with the highest amylolytic activity.

Activation of Isolates

The cultured of LAB and molds amylolytic isolates inocluated in the media MRSA to LAB and PDA to mold with the *Streak plate method*, then incubated at room temperature for 2 x 24 hours for LAB and 3x24 hours for mold. Cultures stock prepared activated for production of inoculum using sterile distilled water.

Isolates that have been activated inoculated into erlenmeyer flask containing distilled water sterilized by taking one tube culture LAB for every 100 ml of distilled sterile while isolates of fungi were inoculated by taking one tube culture mold for every 100 ml of sterile distilled water. The number of cells BAL and mold spores in suspension culture *haemocytometer* calculated to obtain 10^{6} -10^{8} cells / mL for BAL and spores / mL for mold. (Jutono et al, 1993). LAB suspension cultures and molds mixed in one erlenmeyer with a ratio of 1: 1. Furthermore, ready to be inoculated to in the ‘Ragi’ media.

Making ‘Ragi’ inoculum of Mixed Culture of LAB and mould amylolytic

Making ‘Ragi’ substrate that is as much as 1 kg of rice flour and potato flour 1 kg of garlic was added 5g, 5g galangal and lime juice 20 mL and 1 L of boiled water to form dough ‘Ragi” substrate. Inoculated dough with a mixture suspension LAB cultures and molds as much as 10% and incubated for 1 day at a room in a fermentation tank. Furthermore, dried in the sun to form a powder ‘Ragi’. ‘Ragi’ is ready for use as an inoculum in fermentation fresh cassava roots.

Preparation of Substrate Fermentation of Cassava Roots

The substrate to be used as feedstock for fermentation bitter cassava roots in this study is the bitter cassava tubers obtained from Lantongau Village, District Central Mawasangka, Buton Central. Substrate cassava tubers prepared by bark peeled cassava tubers, and then cleaned under running water and then soaked in a 5% salt water for 24 hours, then cut into small parts then dried in the sun. Tubers of cassava bitter dried and then soaked with sterile distilled water for 12 hours, then as many as 1 00 g inserted into fermentor. Furthermore, the substrate in a container sterilized by irradiation with UV for 2 x 45 minutes, Furthermore cassava tubers sterile ready to be inoculated with yeast powder into 1.00 g bitter cassava tubers.

Fermentation Process

Substrates bitter cassava roots inoculated inoculum mixture according to the treatment concentration, namely: 5%, 10%, 15% (v / g) and fermented (incubated) for 0, 2, 4, 6, 8 days at room temperature. Fermented then analyzed its protein content and HCN.

Analysis of Protein and HCN

The method used in measuring levels of the protein in this study using the Biuret method. Rated absorbance is measured using spetrofotometer UV at a wavelength 645 nm. HCN content analysis using argentometry method. Distillate mixture is added to a solution of 0.02 g $AgNO_3$ until turbidity occurs. Volume $AgNO_3$ used for titration up to produce a white precipitate (Sudarmadji, 1984).

Data analysis

Data obtained from each treatment were analyzed by analysis of variance multipath (two-factor). The study consisted of two independent variables, namely the concentration of BAL and mold inoculum mixture amiloltik (5%, 10%, 15%) and fermentation time (0,2,4,6,8) days. Analysis of data used SAS software (Statistical Analysis System.

RESULT AND DISCUSSION

Analysis of Protein Levels Bitter Cassava tubers

Measurement of protein levels bitter cassava tubers done to know how big contribution of BAL and molds amylolytic in increasing the protein content of cassava tubers bitter after fermentation. The measurement of protein content of bitter cassava tubers protein content done using biuret method. Data protein content measurement results are listed in Table 1.

Tabel 1. The protein content of bitter cassava tubers during fermentation

No	Fermentation Time / Inoculum concentration	The protein content of bitter cassava tubers (mg/g)				
		0	**2**	**4**	**6**	**8**
1	K0	35,06	38	50,2	56,95	54,65
2	K1	63,08	64,71	84,07	89,94	88,05
3	K2	88,77	92,75	94,12	100,6	98,30
4	K3	102,28	107,9	126,53	177,28	175

Table 1 shows that the protein content of bitter cassava tubers increased during fermentation. The highest protein levels at day six with the provision of inoculum concentrations as much as 15% compared to other treatments and control (Appendix 8). It is suspected that the treatment granting inoculum concentration of 5% and 10% of the substrate is not proportional to the amount of inoculum, wherein the amount of inoculum small ineffective to hydrolyze a substrate available, ultimately enzyme produced to hydrolyze starch into glucose, is then converted to protein too little. According Sakidja (1989) *in* Yusuf (1996) that as long as the substrate present in sufficient quantities, the reaction rate is directly proportional to the concentration of the

enzyme. However, the reaction rate will rise only up to a certain level and finally the reaction rate becomes fixed, it will even decrease when the number of substrate are not sufficient.

Increased levels of a protein is strongly influenced by the long fermentation time and inoculum concentration. Long fermentation time influenced the protein content of bitter cassava tubers. During the fermentation will cause mold growth and amylolytic BAL, resulting in increased cell mass, eventually the protein content increased, too. During the fermentation process increased LAB and mold growth amylolytic, amylase enzymes produced by these microorganisms can remodel tuber cassava starch into simple compounds as a carbon source for activity and growth. Amylase enzyme is an extracellular enzyme produced in cells and released into the fermentation medium to hydrolyze macromolecular (starch) which was originally insoluble become soluble, so it can be absorbed by the cells (Muhiddin and Munir, 2014; Zubaidah, 2012).

During the fermentation process and molds amylolytic BAL will produce the enzyme amylase that breaks down starch into glucose. Glucose is generated from the metabolism of starch by amylase enzyme will then be used as the base material for the synthesis of proteins. According Varalakshmi *et al.* (2008) *in* Mamangkey (2014), working amylase hydrolyze starch into glucose which is the source of pyruvic acid which acts as the main component for the formation of amino acids.

Results of analysis of variance showed that administration of concentration and fermentation time BAL and mold inoculum mixture amylolytic very significant effect on changes in protein levels bitter cassava tubers. Results of analysis of variance for the effect of concentration and fermentation time BAL and mold inoculum mixture amylolytic to changes in protein levels bitter cassava tubers are listed in Table 2.

Tabel 2. The analysis of variance for the effect of concentration and fermentation time LAB and mold inoculum mixture amylolytic to changes in protein levels bitter cassava tubers

Sources						**F. Table**	
Diversity	**Db**	**Jk**	**Kt**	**F Hit**		**0.05**	**0,01**
Concentration	3	64547.09	21515.70	8286.92	**	2.85	4.34
Times	4	11641.38	2910.35	1120.94	**	2.62	3.86
K*H	12	7508.14	625.68	240.98	**	2.02	2.69
Error	38	98.66	2.60				
Total	59	83797.85					
KK	1.80						

Table 2 shows that treatment administration concentration and fermentation time, inoculum mixture BAL and molds amylolytic very significant effect on changes in protein content of tubers of cassava bitter, so proceed with further test of Duncan to determine significant difference whether or not the effect of concentration and fermentation time, inoculum mixture BAL and amylolytic mold to changes in protein levels bitter cassava tubers. Duncan test results for the variable concentrations of BAL and mold inoculum mixture amylolytic to changes in protein levels bitter cassava tubers are listed in Table

Tabel 3. The results of Duncan test for the effect of concentration and fermentation time BAL and mold inoculum mixture amylolytic to changes in protein levels bitter cassava tubers

	Times				
Inoculum Concentration	**0**	**2**	**4**	**6**	**8**
K3	102,28 as	107,90 ar	126,53 aq	177,28 ap	175,00 ap
K2	88,77 bs	92,75 br	94,12 bq	100,60 bp	98,30 bp
K1	63,08 cs	64,71cr	84,07 cq	89,94 cp	88,05 cp
K0	35,06 ds	38,00 dr	50,20 dq	56,95 dp	54,65 dp

Description: The figures are accompanied by letters that are not the same, are not significantly different at α Duncan test 0.05

Table 11 shows that elevated levels of protein during the fermentation with the provision of treatment concentration and fermentation time LAB and mold inoculum mixture amylolytic each significantly different. The highest protein levels at day six, while on the eighth day decreased levels of protein, but not significantly different protein levels at day six. The results show that the interaction of the highest protein levels with treatment provision K3 inoculum concentration (15%) on the sixth day of fermentation.

Analysis of levels of cyanide acid (HCN) Bitter Cassava Roots

Tubers of cassava bitter are used as raw material for making "Wikau Maombo" nutritional value is high enough, one of which is a carbohydrate. In addition to the nutritional content , tuber of cassava also contains substances antinutrisi namely HCN that can harm human health and can even cause death if consumed in a dose of 0.5 to 3.5 mg / kg body weight. Efforts were made to reduce the toxicity of cyanide in cassava is fermented. the content of HCN can be eliminated or reduced in number by maltreatment is through fermentation. During fermentation will occur breakdown compound linamarin be free cyanide which caused akitivitas enzyme linamaras e. Hydrolysis substance cyanogenic carried out by enzymes linamarase owned microbial, where this enzyme will break bonds of cyanide toxic (Kobawila *et al.* (2005).

Measurements of HCN bitter cassava tubers was conducted to determine the effect of concentration and fermentation time BAL and mold inoculum mixture amylolytic the bitter cassava tubers after fermentation. Measurements of wood bitter tuber tuber HCN done using methods argentometry. Data HCN content measurement results are listed in Table 4.

Table 4. The results of measurements of HCN bitter cassava tubers during fermentation

No	Fermentation Time / oculum ncentration	HCN content of cassava tubers bitter (mg/kg)				
		0	2	4	6	8
1	K0	150,84	126,47	116,75	96,84	95
2	K1	86,76	81	71,28	45,54	42,77
3	K2	43,67	41,94	39,96	33,48	30,89
4	K3	30,24	24,48	20,38	18,47	16,42

Table 4 shows that the levels of HCN bitter cassava tubers decreased during fermentation. HCN levels are the lowest on the eighth day by administering the inoculum concentrations of 15%. Decreased levels of HCN is strongly influenced by the long fermentation time and inoculum concentration. Long time effect on the rate of fermentation and mold growth amylolytic LAB, where the number of BAL and molds that grow will affect amylolytic enzymes produced linamarase. Zubaidah (2012) states the increasing fermentation time, further increasing cell growth also *Aspergillus niger* and *L a ctobacillus plantarum* , so that the resulting enzyme linamarase also increased. The resulting increase in enzymes that might put the ability of the enzyme to degrade compounds linamarin also increased, thereby lowering the HCN content of cassava.

Decreased levels of HCN highest in treatment provision inoculum concentration of 15% (K3) compared to treatment provision inoculum concentration of 5% and 10% and controls. It is alleged that on treatment provision inoculum concentration of 5% and 10% of the substrate is not proportional to the amount of inoculum, wherein the amount of inoculum small ineffective to hydrolyze a substrate available, ultimately enzyme produced to hydrolyze linamarin too little. Increased activity of the enzyme linamarase generated will cause a reshuffle linamarin more op timal, where this enzyme will break bonds HCN toxic, HCN will be broken down into methane and ammonia to be used as a source of N microorganism for protein synthesis (hillocks *et al* ., 2002) *in* (Mamangkey, 2014). this is supported by research Muhiddin and Munir (2014) that a decline HCN content on "Wikau Maombo" during the fermentation process takes place. The lowest in the HCN content "Wikau Maombo" fermented using BAL and *Rhizopus* sp. with concentration Rhizopus inoculum LAB 75% and 25% during the four days of fermentation in the amount of 13.20% to 11.20%.

Measurement data HCN content during fermentation, then analyzed using a completely randomized design (CRD) and HCN content measurement data were analyzed through analysis of variance (ANOVA) using software SAS (*Statistical Analysis System*). If the results show significant differences, Duncan test

was done to determine whether or not the treatment was significantly different administration inoculum concentration and fermentation time to change HCN content of cassava tubers bitter. Results of analysis of variance for the effect of concentration and fermentation time BAL and mold inoculum mixture amylolytic to changes in levels of HCN bitter cassava tubers listed in Table 5

Table 5 . Results of analysis of variance for the effect of inoculum concentration and fermentation time to change HCN content of cassava tubers bitter

Sources Diversity	db	jk	Kt	F Hit		F. Table 0:05	F. Table 0:01
					*		4:34
Concentration	3	78397.79	26132.60	6101.64	*	2.85	
					*		3.86
Day	4	8549.58	2137.40	499.06	*	2.62	
					*		2.69
K * H	12	3476.19	289.68	67.64	*	2:02	
error	38	162.75	4:28				
Total	59	90596.51					
KK	3:41						

Table 5 shows that treatment administration concentration and fermentation time, inoculum mixture BAL and molds amylolytic very significant effect on changes in levels of HCN tuber cassava bitter, so proceed with further test of Duncan to determine significant difference whether or not the effect of concentration and fermentation time, inoculum mixture BAL and amylolytic mold to changing levels of HCN bitter cassava tubers. Duncan test results for the variable concentrations of BAL and mold inoculum mixture amylolytic to changes in protein levels bitter cassava tubers are listed in Table 6.

Table 6. The results of Duncan test for the effect of inoculum concentration and fermentation time BAL and mold inoculum mixture amylolytic to changes in HCN levels bitter cassava roots

Inoculum Concentration	Times 0	2	4	6	8
control	150,84 ap	126,47 aq	116,75 ar	96,84 as	95,00 as
K1	86,76 bp	81 bq	71,28 br	45,54 bs	42,77 bs
K2	43,67 cp	41,94 cq	39,96 cr	33,48 cs	30,89 cs
K3	30,24 dp	24,48 dq	20,38 dr	18,47 ds	16,42 ds

Description: The figures are accompanied by letters that are not the same, are not significantly different at α Duncan test 0.05.

Table 6 shows that the reduction in HCN content during fermentation with the provision of treatment and fermentation time, inoculum concentration mixture of BAL and k Apang amylolytic each significantly different. The results showed that the levels of interaction with the lowest HCN treatment K3 Award inoculum concentration (15%) on the eighth day of fermentation, but not significantly different protein levels at day six. Treatment award K3 inoculum concentration (15%) on the sixth day of fermentation is the most effective treatment in reducing HCN content of cassava tubers bitter.

CONCLUSIONS

Based on the results of the study can be summarized as follows.

1. Concentration and fermentation time BAL and mold inoculum mixture amylolytic significant effect to increase protein content of bitter cassava tubers. The protein content of cassava tubers fermented bitter highest in K3 inoculum concentration (15%) on the sixth day of fermentation, ie 177.28 mg / g.
2. Concentration and fermentation time BAL and mold inoculum mixture amylolytic significant effect on decreasing levels of HCN bitter cassava tubers. HCN content of cassava tubers fermented bitter highest in K3 inoculum concentration (15%) on the sixth day of fermentation, ie 177.28 mg / g.

SUGGESTION

Suggestions put forward in this study is better:

1. To get bitter cassava tubers with increased protein levels and decreased levels of HCN highest, should be fermented for six days with inoculum concentration of 15% for every 100 g bitter cassava tubers.
2. To determine the most effective inoculum concentrations necessary to do research that is equal to the amount of inoculum is more varied.

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