

Preliminary Study of Fermented Pickle of *Tabah* Bamboo Shoot (*Gigantochloa nigrociliata* (Buese) Kurz)

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Abstract—Processing *tabah* bamboo shoot as fermented pickle is one of the way to increase the shelf life of this bamboo shoot. The advantage of this shoot is low concentration of hydro cyanic acid (HCN) make it potential for functional food product. This study aimed to determine the characteristic of *tabah* bamboo shoot pickle such as total of lactic acid bacteria (LAB), pH, total acidity, and hydro cyanic acid (HCN) content, and also find the LAB's type involved during fermentation, and organic acids' profiles. The pickle was made by natural fermentation with 6% salt concentration and fermentation conducted for 13 days.

The result showed during the fermentation time, in the 4th day LAB's number was highest as much as 72×10^7 CFU/ml and the lowest pH was 3.09. We also found decreasing in HCN from 37.8 ppm at the beginning to 20.52 ppm at the end of fermentation process. The organic acids detected during the fermentation were lactic acid with the highest concentration was 0.0546 g/100 g and small amount of acetic acid. By using PCR method, the 18 of LABs which had rod shape were detected as member of *Lactobacillus* spp., in which 17 strains detected as *L. plantarum*.

Keywords—Fermentation, LAB, pickle, *tabah* bamboo shoot.

I. INTRODUCTION

BAMBOO – belongs to *Gramineae* family – grow spread over the tropical, subtropical and temperate climate area. Some bamboos species have high economic value and could process as edible product, such as *Bambusa balcooa*, *Dendrocalamus giganteus*, *D. hamiltonii*, *D. strictus*, *Elocanna baccifera*, *D. sikkimensis*, *Chimonobambusa hookeriana*, *D. longispathus*, *B. tulda*, *Phyllostachys bambusoides*, *D. asper*, *Gigantochloa atter*, *G. robusta*, and *G. nigrociliata* (Buese) Kurz [1]. Various researches about the advances nutritional qualities of bamboo shoots had been conducted. The bamboo shoot has protein, carbohydrates, dietary fiber, low content of fat and cholesterol, vitamins, amino acids, anti-oxidant (flavones, phenols, steroids), and mineral (potassium, phosphorus, sodium, magnesium, and calcium) [1]-[5].

Since the bamboo shoot offers various health benefit likes anti-oxidant, anti-free radical, anti-aging, anti-cancer, prevent

cardiovascular disease, weight loss, to improve digestion, decrease blood pressure, and anti-microbial activity [2], [4], [5], bamboo shoot has been consumed as food items in many countries like China, Japan, US, North East India, Thailand, Nepal, Bhutan, Korea, Australia, New Zealand, Malaysia and Indonesia. The bamboo shoot as edible product in most countries were consumed in the form of raw, dried, canned, boiled, fermented or as medicine [4]. Fermenting bamboo shoot is a traditional method for food preservation. Fermented bamboo shoot had been prepared in many countries for many centuries, *mesu*, *lung-siej*, *soibum*, *soidon* were some kind of fermented bamboo shoot prepared by the ethnic people in the Himalayan region [6], also there are *khoris-tenga*, *amil hendua* (India), *alu tama* (Bhutan), and *naw mai dong* (Thailand) [4]. Those fermented bamboo shoot were produced by fermentation of lactic acid bacteria (LAB).

Gigantochloa nigrociliata (Buese) Kurz - in Bali known as *tabah* bamboo - is the indigenous bamboo species which grows in District of Pupuan, Tabanan, Province of Bali. This edible bamboo shoot has nutritional content as water (92.2%), protein (2.29%), fat (0.23%), carbohydrates (1.68%), and fibers (3.07%). Compared to the others, it has low concentration of hydro cyanic acid (HCN), about 7.97 mg per 100 g raw material makes this bamboo shoot has flat taste [7]. However, as found for almost bamboo shoot, it is characterized by the seasonal availability and perishability in natural condition.

Although the *tabah* bamboo shoot is not so popular as food product, it is important to utilize it to support the food security in the region through food diversification. One way to increase the shelf life of *tabah* bamboo shoot is to process it as fermented pickle with natural fermentation. The fermented pickle can be stored for longer period when the fresh shoot is not available. The present study was carried out to gather information about total of lactic acid bacteria (LAB), pH, total acidity, HCN content, detection of LAB's type involved during fermentation, and organic acids' profiles of fermented pickles of *tabah* bamboo shoot.

II. RESEARCH METHODOLOGY

A. Experimental Design

This study conducted in laboratory. The making process of *tabah* bamboo shoot pickle with natural fermentation is using the Completely Random Design. The salt concentration added is 6% w/v [8] and sugar as per taste. The treatment factor is

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time of fermentation (T), which consists of 6 levels, i.e.: 0, 1, 4, 7, 10, and 13 days.

B. Sample Preparation

The bamboo shoots were weighed 250 g per vessel. The vessels were made of glass. Salt solution which made from water, salt (6% w/v), and sugar as per taste were mixed with the bamboo shoots, then the pickle transferred to a sterile glass vessel. The fermentations were carried out in the room temperature ($27 \pm 2^\circ\text{C}$). Stirred once a day and kept unopened according to the treatment [3] [9, modified method].

Samples were taking at the beginning of fermentation (day 0) and day 1, 4, 7, 10, and 13. For testing type of lactic acid bacteria which present in the pickle, samples were taken at the end of each stage and kept refrigerated at 4°C for analysis. The samples of organic acids were quickly frozen by placing each of them in a plastic container on a freezer. The frozen samples were kept at -36°C until used for further analysis.

C. Determination of Total LAB Count

The total LAB count was determined following the method described in [9]. Ten g sample was blended in 90 ml sterile NaCl solution (0.85% w/v) for 2 minutes and 1:10 dilution was made. The 0.1 ml were plated onto the MRS agar and incubated at 37°C . The colonies appeared on the selected plates (50-300 colonies per plate) were counted as colony forming units (CFU) per ml of the sample.

D. Determination of pH

The pH of the juice sample was determined using pH meter (Toa. IM-405). About 5 g of juice sample was measured by placing drop directly onto the electrode of pH meter.

E. Determination of Titratable Acidity

The titratable acidity expressed as g lactic acid per 100 g sample was measured following modified method of [10]. The standard NaOH 0.1 N was standardized using $(\text{COOH})_2 \cdot 2\text{H}_2\text{O}$ (oxalic acid). For each sampling, 1 g sample was added to Erlenmeyer's flask, followed by addition of 25 ml distilled water. The titration used 0.1 N NaOH and some drops of phenolphthalein.

F. Determination of Hydro cyanic (HCN) Content

The anti-nutrient (cyanogen) content was estimated as hydro cyanic acid (HCN) following method in [10]. About 5 g of each sample was added to beaker glass, followed by addition of 50 ml distilled water then soaked about 2 hours. Again, the 25 ml distilled water added to beaker glass, and then the steam distillation started. The Erlenmeyer flask with 20 ml of 2.5% NaOH was prepared to store the distillate. The distillation process stopped after the distillate reached 75 ml. Finally, the distillate was added by 4 ml NH_4OH and 2 ml KI 5%, before titrated by AgNO_3 0.02 N until the distillate turbid.

G. Organic Acid Profile

1) Sample Preparation for Organic Acids Analysis

The frozen samples were thawed at room temperature and 5 g of each sample was diluted with 25 ml distilled water.

Homogenized about 15 minutes and filtered using Whatman paper. About 1 ml brine was pipetted and transferred into a centrifuge tube. The samples were added with 10 μl sulfo-salicylic acid to precipitate the protein, then centrifuged (MSE Microcentaur) at 13,000 rpm for 3 minutes. The supernatant in the centrifuge tube was filtered through a 45 μm filter and 10 μl of the filtrate was injected in the HPLC column.

2) Analysis of Organic Acids

The organic acid were analyzed following ICI methods [11] using the HPLC-apparatus (ICI Instrument), consisting of HPLC gradient controller (ICI Instrument Kortec K45), HPLC pump (ICI Instrument Kortec K25), solvent delivery (Varian pro star), ICI Organic Acid Coloumn 0.65 x 30 cm, ICI cation-exchange the hydrogen form, HPLC automatic injector (ICI Instrument AS 2000), HPLC monitor (Shimadzu RF-535), HPLC UV detector (ICI Instrument Kortec K95), interpreter (ICI Instrument DP 800 data interface, and Shimadzu C-R6A Chromatopac). Acts as the mobile phase is H_2SO_4 0.01N (pH 2.3). The flow rate was set to 0.4 ml/minute; wavelength of UV detector is 210 nm and the column temperature 30°C . The standard used was mixed standard in 100 mM concentration, contain of lactic acid, acetic acid, propionic acid, and butyric acid. Standard curves were plotted using peak area given by ICI Chromatopac. The run program of HPLC is isocratic. Analysis of each sample took time about 25 – 30 minutes.

H. Isolation of Lactic Acid Bacteria (LAB)

Determination of LABs type from the pickle is conducted by using serial dilution-agar plate technique. The media used were de Man Rogosa Sharpe (MRS) Broth (Oxoid CMO359) and MRS agar (Oxoid CMO361) which contain peptone 10.0, lab-lemco' powder 8.0, yeast extract 4.0, glucose 20.0, sorbitan mono-oleate 1 ml, di-potassium hydrogen phosphate 2.0, sodium acetate $3\text{H}_2\text{O}$ 5.0, tri-ammonium citrate 2.0, magnesium sulphate $7\text{H}_2\text{O}$ 0.2, and manganese sulphate $4\text{H}_2\text{O}$ 0.05.

In serial dilution-agar plate technique [12], 10 g of juice samples were suspended in 90 ml sterile 0.85% NaCl (to make the total volume to 100 ml) and mixed it well to form a microbial suspension. Serial dilutions of 10^{-2} , 10^{-3} , 10^{-4} , and 10^{-5} were made by pipetting 1 ml to 9 ml sterile 0.85% NaCl. About 0.1 ml suspension of each dilution is spread on the agar plates, incubated at 37°C for 48 hours. The colonies from 10^{-5} spread plates were randomly selected using sterile toothpicks and transferred into the 5 ml MRS Broth and incubated at 37°C for 24 hours. The only colonies with clear zone around them were taken.

The LAB were purified using streak plate method by transferring on MRS agar and incubating at 37°C for 48 hours. The pure colonies were streaked on MRS agar, incubated at 37°C for 48 hours and the single colonies from each streak plates were selected and transferred into 5 ml MRS Broth. All of the strains were initially examined to the gram staining and catalase test. Only bacteria with Gram positive and Catalase negative were collected. The selected strains were maintained with sterilized 30% glycerol oil and kept in freezer at -36°C .

I. Molecular Identification of LAB

Molecular identification of LAB is conducted by using PCR (Verity 96 Well Thermal Cycler Applied Biosystem) according to method from Cho et al. [13]. For the preliminary study, the LAB identified was specific for the *Lactobacillus*

spp. The primer used were *Lactobacillus*-specific primer set, Lacto 16S-F/Lacto 16S-R [22], Lbr-F/Lbr-R as forward and reverse primer for *L. brevis*, and Lpl-F/Lpl-R which as forward and reverse primer for *L. plantarum* [13] obtained from Genetika Science Jakarta (Table I).

TABLE I
LIST OF OLIGONUCLEOTIDE PRIMERS USED IN THIS STUDY

Primer Name	Primer sequences (5' → 3')	Species/Genes	Product Size (bp)
Lacto16S-F	GGA ATC TTC CAC AAT GGA CG	<i>Lactobacillus</i> spp.	216
Lacto16S-R	CGC TTT ACG CCC AAT AAA TCC GG		
Lbr-F	AAA GCC AGG TAC TGT TAA GGG TGC	<i>L. brevis</i>	482
Lbr-R	AAC CAT CAG CCT TAG TGT CAG CG		
Lpl-F	AAG GCC GTA GTC AGT CGT CTA TGG	<i>L. plantarum</i>	313
Lpl-R	TCA ACCA CAC GAA TAT CAG CCG G		

Sequence similarity of the oligonucleotide for specific primer was estimated by searching the homology in the Genbank DNA database using BLAST. For the positive control, LAB strains used were *L. brevis* FNCC 0021 and *L. plantarum* FHCC 0027 (supplied by Food and Nutrition Department, Gajah Mada University Yogyakarta).

J. LAB Isolates Genomic DNA Extraction

The DNA of LAB isolates was extracted from overnight culture of bacteria isolates using Bacterial Genomic DNA extraction kit (Roche High Pure PCR Template Preparation Kit Version 20). The PCR reaction mixture (10µl) consisting of 5µl PCR master mix, 0.5µl Primer F 10µM, 0.5µl Primer R 10µM, 2µl H₂O, and 2µl DNA Template. The single PCR program was as follows: Predenaturation was performed at 95°C for 5 minutes and the target DNA was amplified in 35 cycles. Each cycle consisted of denaturation (94°C for 30 sec), annealing (58°C for 1 min), and extension (72°C for 2 mins). The final extension was performed at 72°C for 10 mins [13]. The PCR product were analyzed by electrophoresis (BioRad) on a 1.5% agarose gel stained with ethidium bromide in 1x TBE buffer at 65 V, 400 mA for 30 mins. The sizes of DNA fragments were estimated using a marker 100 bp DNA ladder (Invitrogen). The bands were visualized and documented using the gel documentation apparatus (GelDoc XR BioRad).

III. RESULTS

A. Total LAB

The duration of fermentation i.e. 0, 1, 4, 7, 10, and 13 days were analyzed for total lactic acid bacteria (LAB). Fig. 1 showed the total LAB count trend of bamboo shoot pickle.

During the fermentation of bamboo shoot pickle, naturally the occurrence of lactic acid bacteria was found varies. After 24 hours the cells have rapidly grown until day 4th where the highest number as much as 72×10^7 CFU/ml. However, at day 7th the cell number was decline, but on day 10th the LAB number rise to 21.50×10^7 CFU/ml. Until the end period of fermentation (day 13), the LAB count was 5.78×10^7 CFU/ml.

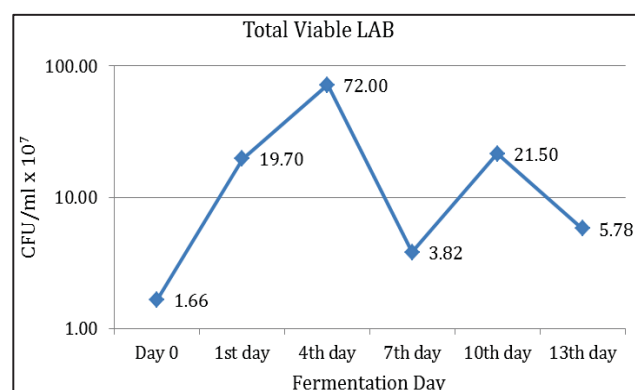


Fig. 1 Total LAB Count during Fermentation

B. pH and Titrable Acidity

The pH value ranged between 5.01 and 3.09. The initial pH on the day 0 was 5.01, and then after 24 h dropped to 3.76, until reach the lowest pH (3.09) on the day 4. On the day 7 and 10 the pH was rise to 3.35 and 3.50 then decline to 3.32 on the end period of fermentation (Fig. 2).

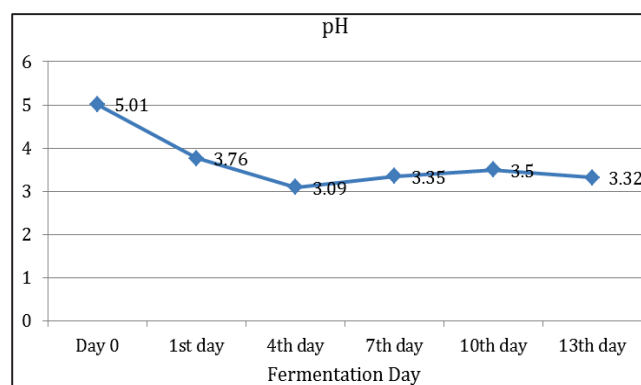


Fig. 2 pH Trend of Tabah Bamboo Shoot Pickle

Meanwhile, total acidity which expressed as lactic acid generally tends to increase. It ranged between 0.19 g/100 g and 0.39 g/100 g. At the beginning of fermentation, total acidity was 0.19 g/100 g and it's rising during the fermentation period. The highest of total acidity was 0.39

g/100g reached at day 13 (Fig. 3). The result showed that the change trend of LAB number has positive correlation with the change trend of pH and total acidity. The dropped pH indicates the increase of LAB number and total acidity. In optimal environment, the LAB start the activity to change substrates into organic acids and causing the lower pH.

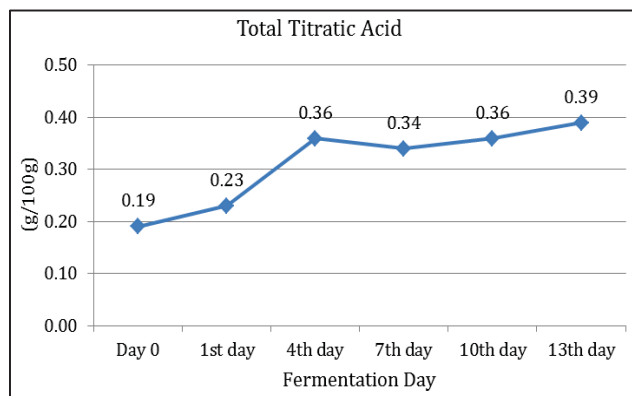


Fig. 3 Total Acidity of *Tabah* Bamboo Shoot Pickle

C. Hydro cyanic Acid

The anti-nutrient (cyanogen) content of fermented pickle which expressed as hydro cyanic acid (HCN) tends to decrease during the fermentation. The highest concentration was 37.8 ppm on the day 0 and the lowest was 20.52 ppm on the day 13 (Fig. 4). According to Soetrisno et al. [14], the hydro cyanic acid of bamboo shoots after steamed process was 76.9 ppm. Putra [15]) also reported that the hydro cyanic acid content of *tabah* bamboo shoot after steamed or boiled were 115.8 ppm. This result showed that the fermentation process of pickle could reduce the hydro cyanic acid content. LAB had important role in reducing HCN and make pickle safe for consumption. Obilie et al. (2003) in [16] reported the groups of lactic acid bacteria such as *L. plantarum* could reduce HCN after 72 hours of fermentation.

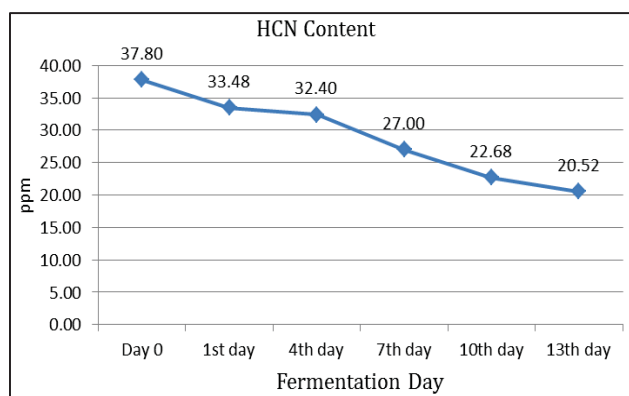


Fig. 4 Trend of HCN Content of *Tabah* Bamboo Shoot Pickle

D. Isolation and Molecular Detection of LAB by PCR

About 48 strains of indigenous LAB isolated from the pickle which belong to positive gram, negative catalase and homofermentative. Eight teen out of these strains were found

has rod shape, and the rests were cocci. In this study, the LABs which identify only LABs with rod shape. The molecular identification was conducted using species-specific PCR. From the analysis, 18 strains were the member of *Lactobacillus* spp. (216 bp PCR product), which consist of 17 strains identified as *L. plantarum* (313 bp PCR product) (Fig. 5)

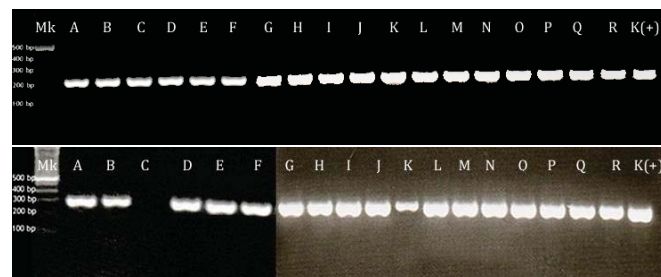


Fig. 5 PCR with Specific Primer for *Lactobacillus* spp. (top) and *Lactobacillus plantarum* (bottom)

Lane: M_k: marker; **A**: PH014ak; **B**: PH014ab; **B**: PH18bb; **D**: PH125bb; **E**: PH126bk; **F**: PH126bs; **G**: PH14as; **H**: PH126ab; **I**: PH19bs; **J**: PH125bk; **K**: PH416bb; **L**: PH714ak; **M**: PH19bb; **N**: PH18bk; **O**: PH15ak; **P**: PH126 as; **Q**: PH714ab; **R**: PH15ab; **K(+)**: positive control (*L. plantarum* FHCC 0027)

Previous research reported LAB from member of *Lactobacillus*, especially *L. plantarum* was occur and play important role in fermentation of bamboo shoot such as *mesu* [9], *jiang sun* [12], and *naw mai dong* [4].

E. Organic Acid Profiles

The organic acids content were analyzed using the HPLC apparatus. The standard acid was standard mix 100 mM content lactic acid, acetic acid, propionic acid, and butyric acid. The retention time of the organic acids was i.e. 10.917 mins. (lactic acid), 12.067 mins. (acetic acid), 14.112 mins. (propionic acid), and 17.22 mins. (butyric acid).

The organic acids which detected in bamboo shoot pickle during the fermentation were varied. At the day 0 none of the targeted acid was detected. At day 1 and day 4, we detected lactic acid. At day 7, two organic acids i.e. lactic acid with the decrease content than day 1 and 4, and little number of acetic acid were detected. At day 10 and 13, only acetic acid with increasing number than day 7 was detected and the lactic acid was not. The organic acid profile of bamboo shoot pickle during the fermentation showed in Table II.

The organic acids detected after 24 hours of fermentation. On the day 0, production of organic acids was not detected. However, at the 1st day lactic acid increase rapidly till reach the highest concentration (0.0546 g/100 g) during the fermentation before decreasing. On the 4th day the concentration of lactic acid decline until disappear on the day 10. The decreasing of lactic acid on 7th day was followed by acetic acid detection. Although the acetic acid detected only with the small amount, but its concentration increase till the day 13 of fermentation (Fig. 6).

TABLE II
 THE ORGANIC ACID OF BAMBOO SHOOT PICKLE

Fermentation Time (day)	Time (minutes)	Area	Name
0	5.432	26082	Unknown
1	5.390	34276	Unknown
	10.940	350	Lactic acid
4	5.365	38485	Unknown
	10.937	234	Lactic acid
7	5.378	30508	Unknown
	10.915	184	Lactic acid
10	12.013	15	Acetic acid
	5.350	15062	Unknown
13	12.125	20	Acetic acid
	5.442	47737	Unknown
	12.017	175	Acetic acid

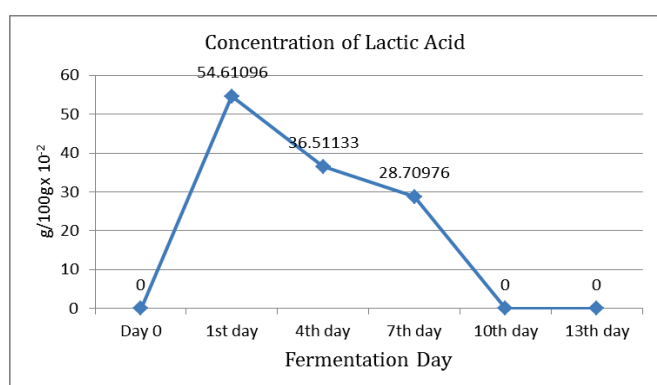


Fig. 6 Concentration of Lactic Acid during Fermentation

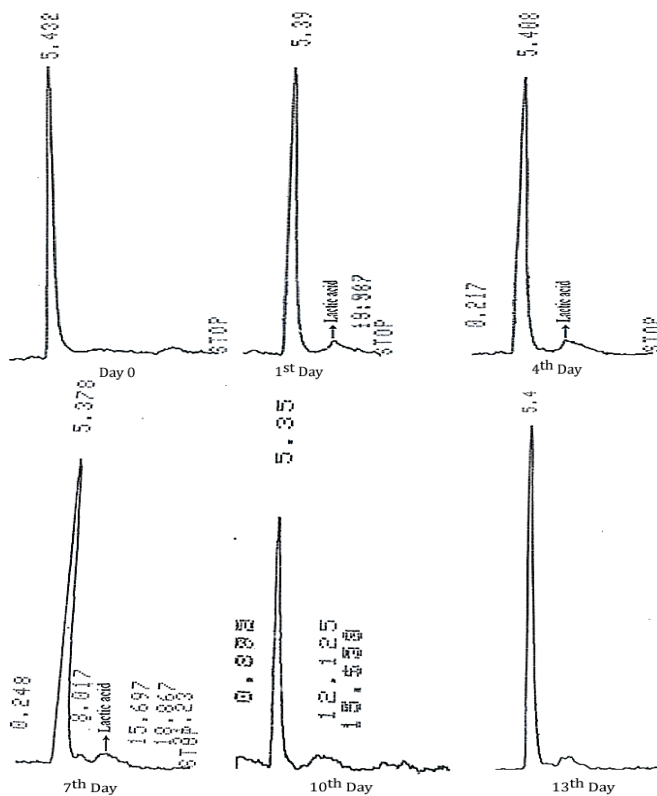


Fig. 7 Organic Acid Profile of Tabah Bamboo Shoot Pickle

The decline concentration of lactic acid might be caused by the degradation of lactic acid to acetic acid. The lactic acid conversion rate was occurring in low pH condition. Elferink et al. [17] reported some of Lactobacillus species were able to carry out anaerobic lactic acid degradation. The degradation was influenced by environmental conditions, such as pH and temperature.

Organic acids profile showed on the day 0 none of the targeted peak was detected. At the 1st, 4th, and 7th days were detected peak with the retention time which identified as lactic acid. On the day 7, in addition to lactic acid, data also showed retention time of acetic acid. However, in the profile, this peak was not clear because it has very small area. On the day 10 and 13, the peak of lactic acid was disappear (Fig. 7).

IV. DISCUSSION

Fermentation is the traditional method for preserving the food. The LAB fermentation in *tabah* bamboo shoot pickle could preserve the raw material from the rapid degradation process. Naturally the existence of LAB in pickle was found to vary during the fermentation. Result showed that the change trend of LAB number has positive correlation with pH and total acidity. In the optimal condition, after 24 hours of fermentation, the cell growth rapidly which initiated the indigenous LAB enter the exponential phase. The LAB's growth continued until the highest of cell number (72×10^7 CFU/ml) recorded on the 4th day. An increase in cell number could lower the pH until the lowest value (3.09) reached on the 4th day. This is an indication that the LAB responsible in the activity for changes substrates into organic acid.

According to Holzapfel [18]; Leroy and De Vuyst (2004) in [19], the LAB has important role in the fermentation of pickle because they provide a rapid acid accumulation in the raw material with the production of several organic acids. Tamang and Sarkar [9] also reported that during the fermentation, some species of LAB produced lactic acid and acetic acid, which reduce pH of the substrates making the products more acidic in nature, and inhibit the growth of pathogenic microorganism, thus food are safe for consumption. According to Foo et al. [20], the increase of biomass cell and organic acids productions are the main reason for pH reduction in fermented food. After the exponential phase on the 4th day, the cells grow slowly until the end period of fermentation, due to the continuously falling of nutrient concentration [21].

V. CONCLUSION

In conclusion about the present study, fermentation of *tabah* bamboo shoot using 6% salt concentration in natural fermentation had some unique characteristics. LAB which naturally occur in raw material continue the lactic acid fermentation and manage the acidic environment by produce lactic acid, small amount of acetic acid, and lower pH. The fermentation also decrease hydro cyanide content (20.52 ppm). The total of indigenous LABs isolated from the pickle i.e. 48 strains, and we found 18 out of these had rod shape. All of the LAB with rod shape were detected as member of

Lactobacillus spp., in which 17 strains identified as *L. plantarum*.

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