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# Optimization of White Radish Fermentation with Rice Bran

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**Abstract:** *Asian white radish is a popular root vegetable in asia. It can be consummed as fresh, dried, salted and pickle products. Japanese pickle vegetable (nukazuke) fermented in rice bran bed. There is no research related to this fermentation in Cambodia. Therefore, the ojective of this study is to optimize the white radish fermentation wtith rice bran. In this study, the physicochemical composition in white radish and rice bran were analyzed such as pH, moisture (%), carbohydrate (%), protein (%), acidity (%), fiber (%) and ash (%). Moreover, ratio variation, salt variation, size variation and drying and without drying white radish were conducted for three days fermentation at room temperature in order to determine the optimum condition. Fermented rice bran was withdrawn during fermentation at day0 to day3 for analysis of moisture, pH and salt (%). At the end of fermentation, the juice of fermented white radish was also analyzed for moisture, pH, salt (%) and total acidity. In addition, fermented rice bran was identified LAB during fermentation and lactic acid was conducted by HPLC with fermented rice bran and white radish juice with salt variation. The result demonstrated that fermented white radish contained the pH value of 4.08-4.26, moisture content of 85.00%-88.87%, salt concentration of 4.5%-4.7% and total acidity of 0.17%-0.19%. This fermentation was homo-fermentation. The optimum fermentation conditions was ratio water and rice bran 1:1, 5% of salt concentration, size 1/2 and with drying of white radish.*

**Keywords:** White radish; Rice bran; Pickle vegetable fermented; Lactic acid fermentation; Homo-fermentation.

## 1. INTRODUCTION

Asian white radish is a popular root vegetable throughout Asia. It can be consumed as fresh, dried, salted and pickled product (Coogan and Wills, 2002). In Cambodia, the Cambodian people can only make Chhaypouv by mixing white radish with salt and drying. This technique has been passed down from generation to generation. There are another type of vegetable fermentation in Japan called nukazuke. It is a type of Japanese pickle vegetable fermentation in rice bran bed. Nukazuke is very popular in Japan due to its crunchy texture, delicious pickle and original color is preserved. Most of edible vegetable are fermented by this method, included white radish, cabbage, cucumber, eggplant and ginger (Luchi *et al*., 2012). So far, Cambodian people do not know how to ferment white radish by using this fermentation technique and have limited information about this type of fermentation.

The objective of this research was therefore to optimize the white radish fermentation with rice bran by changing the ratio between water and rice bran, the salt concentration, the size of white radish, and drying of white radish. And the rice bran that is available in Cambodia was used in this study.

## 2. METHODOLOGY

### *2.1 Raw materials*

5kg of fresh white radish were bought from Neakmeas Market in Phnom Penh for fermentation. The good white radish were chosen with no spots or any black. For rice bran, it was bought from rice milling machine at Prekhsay commune, Peamror district, Preyveng province. The pure rice bran and fine, not the husk was chosen. The physico-chemical characteristics of fresh white radish and rice bran were first determined. And non-iodine salt was bought from Brampimakara market in Phnom Penh for fermentation.

### *2.2 White radish fermentation*

The fermentation of white radish with roasted rice bran was conducted with white radish and pure rice bran in Cambodia.

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Four experiments were conducted fermentation for three days in room temperature. During and end of fermentation, fermented rice bran was withdrawn at regular interval at 0 h, 24 h, 48 h and 72 h for physicochemical analysis on moisture content, pH value and salt concentration. In addition, the juice extract from fermented white radish underwent analysis on moisture content, pH value, salt concentration and total acidity on the last day of fermentation.

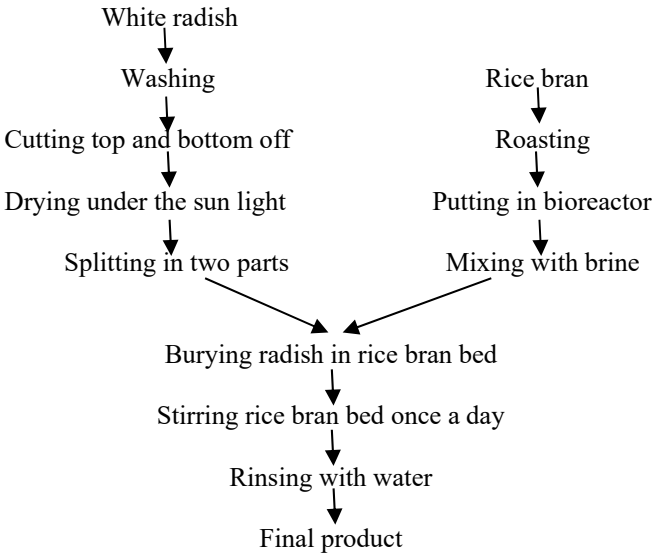



Fig. 1. Schema of fermentation

*2.2.1 Ratio variation*

This fermentation was prepared in 5 different ratios of water and rice bran. The following proportions: 0.7:1, 0.8:1, 0.9:1, 1:1 and 1.1:1 were fermented with 100g of salt.

*2.2.2 Salt variation*

The salt solution was prepared in 5 different concentrations comprising 0%, 2.5%, 5%, 7.5% and 10% for fermentation with 1kg of rice bran and 1L of water.

*2.2.3 Size variation*

The fermentation with 100g of salt, 1L of water and 1kg of rice bran were conducted in 3 different sizes (size 1, size 1/2, size 1/4).

*2.2.4 Fresh and dried radish*

In this step of fermentation with 100g of salt, 1L of water and 1Kg of rice bran were conducted with fresh and dried radish.

*2.3 Analysis methods*

The pH values and salt concentrations were measured by pH-meter and salt-meter, respectively. Moisture content was measured by drying in the oven at 105 $^{o}$C for 24 hours. The number of lactic acid bacteria was counted by growing them on MRS agar incubated at 37 $^{o}$C for 24 hours. Lactic acid concentration was measured by HPLC.

## 3. RESULTS AND DISCUSSION

*3.1 Composition of white radish*

The results of composition were not much different between fresh and dried radishes as shown in Table 1. The moisture content was about 94%. The carbohydrate was about 3.2% allowing lactic acid bacteria (LAB) to convert into lactic acid (LA) during fermentation. The protein was only 0.70% and the smallest amount was lipid, aroud 0.02%. For fiber were 0.80% and 1.06% for fresh and dried radish. Ash were 0.76% and 0.93% respectively. Comparing with the results done by Singh and Singh (2013), the composition in white radish in their study were found almost similar to this study. The moisture was 94%, carbohydrate was 3.4%, fiber was 0.8% and protein was 0.7% in their study.

Table 1. Composition of white radish

| Composition | Fresh radish (%) | Dried radish (%) |
|---|---|---|
| Moisture | 94.54 ± 0.03 | 94.13 ± 0.20 |
| Carbohydrate | 3.21 ± 0.00 | 3.16 ± 0.08 |
| Protein | 0.67 ± 0.00 | 0.70 ± 0.00 |
| Lipid | 0.02 ± 0.00 | 0.01 ± 0.00 |
| Fiber | 0.80 ± 0.03 | 1.06 ± 0.03 |
| Ash | 0.76 ± 0.02 | 0.93 ± 0.03 |

*3.2 Composition of rice bran*

According to Table 2, the carbohydrate containing in rice bran was 37.38%. This amount of carbohydrate allowed LAB to convert into LA during fermentation. The moisture content was nearly 10%, protein was about 10%, lipid was 8% and ash was around 9%. Comparing with the results done by Ono *et al*. (2014), the carbohydrate of nukadoko (rice bran bed for pickling) was about 48%, moisture was around 13%, protein was 12% and fat was 18%.

Table 2. Composition of rice bran

| Composition | Rice bran (%) |
|---|---|
| Moiture | 9.78 ± 0.13 |
| Carbohydrate | 37.38 ± 1.18 |
| Lipid | 8.16 ± 0.37 |
| Protein | 10.58 ± 0.17 |
| Ash | 8.89 ± 0.13 |

*3.3 Changing of moisture content, pH value and salt concentration in fermented rice bran with ratio variation*

The highest moisture content was ratio 1.1:1, about 50% at day 0 because more water was added (Figure 2). However, the ratio 0.7:1 had the lowest moisture content, about 40% at day 0. The moisture content for all samples increased highly about 5% at day 1. Meanwhile, the moisture content for all samples on the day 2 and day 3 were increased moderately about 2%. The changing of moisture content during three days of fermentation of rice bran increased about 6% to 9% for all samples. According to the result done by Oliveira *et al.* (2010), the initial moisture content in their study was 50% without vegetable. The moisture content at 0 h was 47.9%, at 24 h was 46.3%, at 48 h was 42.1% and at 72 h was 42.5%.

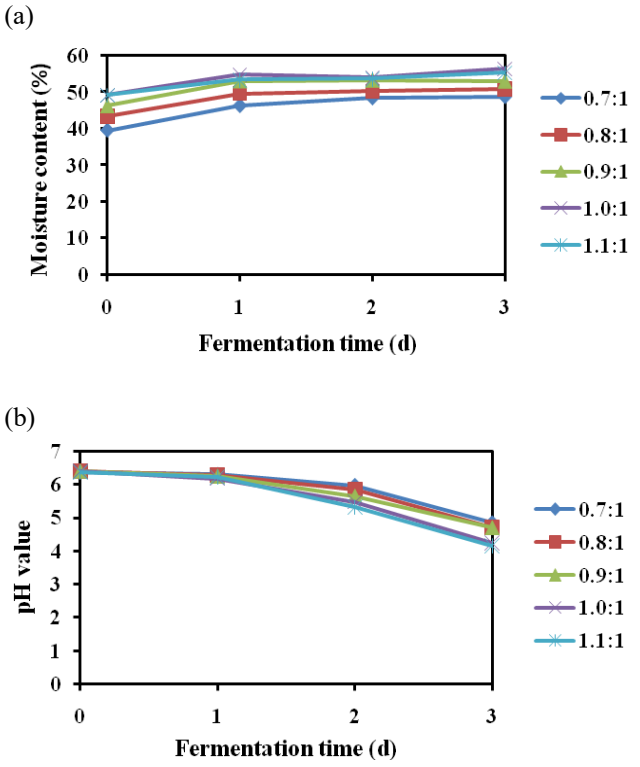



Fig. 2. Changing moisture content and pH value in fermented rice bran with ratio variation

The pH value at the beginning of fermentation for all rice bran samples was approximately pH 6.4. The changing of pH value was almost similar for all fermennted samples by dropping from pH about 6.4 to pH 4.5 during three days. Whereas, the result of pH of fermented rice bran in the previous study showed that the initial pH value was 6.5 and decreased to between 4.0 to 4.2 within 2-3 weeks (Ono *et al.*, 2014).

Among five samples, the ratio 0.7:1 had highest salt concentration of about 5.06% at day 0. However the ratio 1.1:1 had the lowest salt concentration of about 4.03% at the start of fermentation due to higher water content in this sample. Besides this, the salt concentration of all samples was dropped significantly at day1, but the ratio 1.1:1 was slightly dropped from 4.03% to 3.80% of salt concentration because only little water was came out from the white radish and less salt was entered the pulp of white radish. Additionally, at the last day of fermentation, the salt concentration for all fermented samples did not change significantly. The higher concentration of salt was the ratio 0.7:1, about 4.5 % and the lowest concentration of salt was the ratio 1.1:1, about 3.43% (data not shown).

*3.4 Changing of moisture content, pH value and salt concentration in fermented rice bran with salt variation*

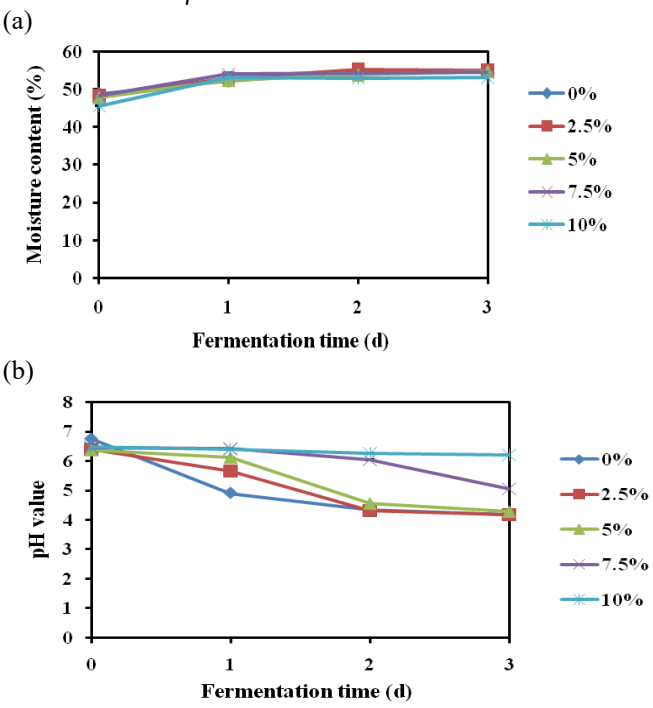



Fig. 3. Changing moisture content and pH value in fermented rice bran with salt variation

The moisture content at the first day of fermentation for most rice bran samples was approximately 48% but the

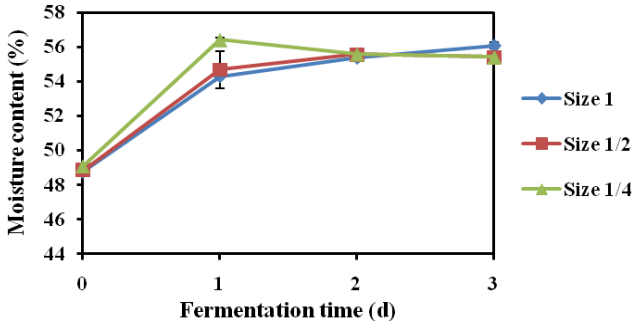
moisture content of 10% sample was about 46%. As for the day 1 of fermentation, the moisture content for all rice bran samples increased significantly to around 53%. However, there was a little rise of moisture content at day 2 and day 3 and it seems to be in equilibrium condition.

The pH value of 5 samples during fermentation was different when different salt concentrations were used. At the first day of fermentation, the pH value was almost the same for all samples at around a pH of 6.5. The pH of the 10% sample remained stable during three days of fermentation at about pH of 6 because of the high amount of salt added. Conversely, the pH value of the 0% sample decreased significantly at the first day of fermentation because no was salt added, which lead to fast reduction in pH value. Meanwhile, the pH value of the 5% sample reduced slightly to a pH of 6 on the first day. Additionally, the pH of the 0% and 2.5% samples dropped to a pH around 4.5 on the third day. On the other hand, the 7.5% sample had no changed in pH the first day of fermentation, with a slight drop in pH on the last day to 5. Comparing with fermented olives, the pH value of final brine was varied between 4.0-4.5 (Montet *et al.*, 2014).

The 10% sample had the highest salt concentration of 9.26% at the beginning of fermentation. At the first and second days, the salt concentration dropped moderately to 7.5% and remained stable at the last day because of equilibrium of salt concentration. Regarding to 7.5% sample, the 7% salt concentration from day 0 fell to 6 % at day 1. The 5% sample had a salt concentration of 5.26% at the beginning of fermentation. The salt concentration increased to 4.26% during the second and third day because the equilibration of salt concentration in white radish and rice bran(data not shown). Comparing with fermented olives, the salt concentration of final brine was varied from 6% to 10% (Montet *et al*., 2014).

*3.5 Changing of moisture content, pH value and salt concentration in fermented rice bran with size variation*

(a)




(b)

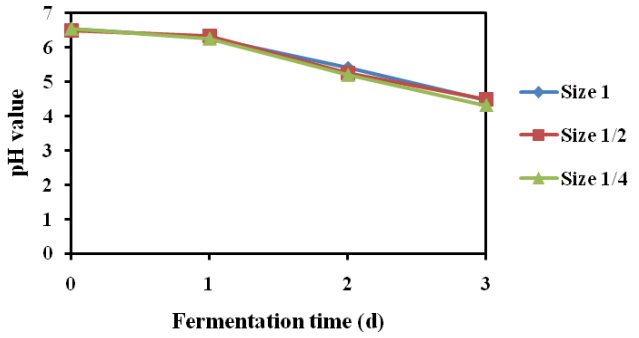



Fig. 4. Changing of moisture content and pH value in fermented rice bran with size variation

The moisture content of all three samples was approximately 49% at the beginning of fermentation. However, although there was a sharp increase in first day of all samples, the most significant growth was sample Size 1/4 with 56.5% smaller piece of white radish were shown to produce more water. The moisture content of the sample Size 1 and sample Size 1/2 had a slight increase during two last days of approximately 55%. The moisture content of the sample Size 1/4 dropped slightly during day 2 and day 3 about 55% as a result of the equilibrium pressure reaction of water in rice bran and white radish.

The pH value of each sample was dropped in the same approximate value during three day fermentation because of using the same amount of water and salt in all three conditions of fermentation. The pH value at the start day was a pH of 6.5. Following a continuous decrease in the pH value, the end result was a significant decrease in pH to 4.4. Comparing to the result of pH of nukadoko indicated that a significant increases the lactate concentration and the pH was rapidly decreased from 6.4 to 4.6 in the first 2 days. The pH decreased slowly from 6.4 to 4.3 during day 2 to day 8. The result in this study was similar to their study (Nakayama *et al.*, 2007).

The changing of salt concentration in fermented rice bran during three days. The salt concentration was approximately 5% at the beginning of fermentation for all samples. Moreover, the salt concentration at the first day fell dramatically to 4% for all three samples (data not shown). Comparing with fermented eggplants, the salt concentration of brine was not exceeding 6% (Montet *et al*., 2014).

*3.6 Changing of moisture content, pH value and salt concentration in fermented rice bran with and without drying of white radish*

The moisture content in fermented rice bran of all sample were very similar, from the beginning of fermentation until the last day of fermentation. The moisture content was approximately 48% at the start day. Interestingly, the moisture content had a steady growth to 54% at the first day.

Water released from the white radish created more water in rice bran bed. The moisture content was increased slightly to around 54.5% during the second and the third day. As a result of, the equilibrium pressure reaction of water in rice bran and white radish at the last day of fermentation.

pH value in fermented rice bran of all samples was nearly the same from the start day of fermentation until the last day. The pH value was approximately 6.4 at the day 0 of fermentation, falling at day 1 to 6.1. However, the pH value decreased sharply to pH 4.5 on day 2 of fermentation because of LAB produced more lactic acid. At the end of day, the pH value dropped slightly to approximately pH 4.3. Comparing with the result done by Sakamoto *et al.* (2011), their study showed that there was a rapid decrease in pH in the first 2 days due to *Lb. numuensis* that were fast grown during this period in nukadoko.

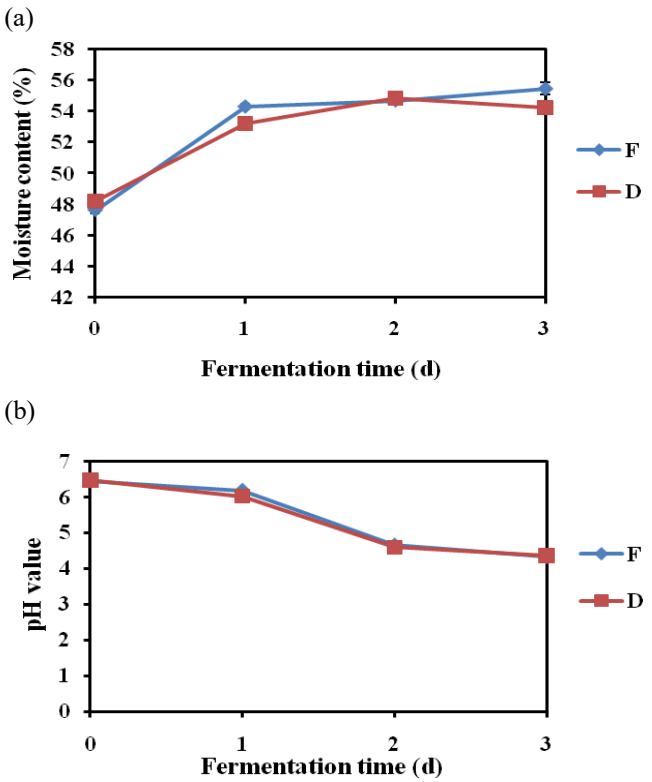



Fig. 5. Changing of moisture content and pH value in fermented rice bran with and without drying white radish

The salt concentration in fermented rice bran for all samples was similar during three day fermentation. The initial salt concentration was around 5.5% for all samples. The salt concentration decreased significantly to about 4.5% at the first day, due to the osmotic pressure between rice bran bed and white radish. The salt concentration dropped gradually approximately 4.3 % during two last days (data not shown).

### *3.7 Physicochimical parameters in fermented white radish with ratio variation*

From Table 3, the moisture content of sample with ratio 1.1:1 was higher due to the higher water contained. The moisture content of samples 0.7:1, 0.8:1 and 0.9:1 were not significant different (p-value > 0.05). On the other hand, the salt concentration and pH value in the sample with ratio 0.7:1 were higher than the others because this sample had a smaller amount of water. In term of pH, pH values of all samples were significant different (p-value < 0.05). Depending on the sensory test, fermented white radish with ratio 1:1 was better than the others due to preferred texture and color. Comparing with the result of physicochemical of long term fermented kimchi done by Nam *et al.* (2007), the salt concentration and pH were lower and the acidity was higher than this study because of long fermentation time. The salt concentration, pH value and total acidity were 3 ± 0.5%, 4.0 ± 0.2% and 1.88 ± 0.76%, respectively.

Table 3. Physicochemical analysis in fermented white radish with ratio variation

| Ratio | pH value | Moisture (%) | Salt (%) | Acidity (%) |
|---|---|---|---|---|
| 0.7:1 | $4.67 \pm 0.00^{a}$ | $82.30 \pm 0.10^{a}$ | $5.0 \pm 0.00$ | $0.14 \pm 0.00^{a}$ |
| 0.8:1 | $4.54 \pm 0.00^{b}$ | $82.10 \pm 0.12^{a}$ | $4.9 \pm 0.00$ | $0.15 \pm 0.00^{a}$ |
| 0.9:1 | $4.36 \pm 0.00^{c}$ | $82.60 \pm 0.67^{a}$ | $4.7 \pm 0.00$ | $0.17 \pm 0.00^{b}$ |
| 1:1 | $4.16 \pm 0.00^{d}$ | $84.42 \pm 0.10^{b}$ | $4.6 \pm 0.00$ | $0.17 \pm 0.01^{bc}$ |
| 1.1:1 | $4.08 \pm 0.00^{e}$ | $84.30 \pm 0.06^{b}$ | $4.5 \pm 0.00$ | $0.19 \pm 0.00^{c}$ |

Different letter in column, the sample are significant different (p-value < 0.05),

Same letter in column, the sample are not significant different (p-value > 0.05),

### *3.8 Physicochemical parameters in fermented white radish with salt variation*

Table 4. Physicochemical analysis in fermented white radish salt variation

| Salt conc. (%) | pH value | Moisture (%) | Salt (%) | Acidity (%) |
|---|---|---|---|---|
| 0 | $4.05 \pm 0.00^{a}$ | $90.04 \pm 0.05^{a}$ | $0.85 \pm 0.01^{a}$ | $0.24 \pm 0.00^{a}$ |
| 2.5 | $3.98 \pm 0.00^{a}$ | $87.94 \pm 0.14^{b}$ | $2.71 \pm 0.02^{b}$ | $0.23 \pm 0.00^{b}$ |
| 5 | $4.08 \pm 0.10^{a}$ | $85.00 \pm 0.14^{c}$ | $4.50 \pm 0.00^{c}$ | $0.19 \pm 0.00^{c}$ |
| 7.5 | $4.57 \pm 0.00^{b}$ | $83.92 \pm 0.06^{d}$ | $6.13 \pm 0.06^{d}$ | $0.13 \pm 0.00^{d}$ |
| 10 | $5.48 \pm 0.00^{c}$ | $80.10 \pm 0.10^{e}$ | $7.67 \pm 0.05^{e}$ | $0.05 \pm 0.00^{e}$ |

DifferenT letter in column, the sample are significant different (p-value < 0.05),

Same letter in column, the sample are not significant different (p-value > 0.05),

According to Table 4., the moisture content of the 0% sample was higher due to a lack of salt. The salt concentration and pH value in the 10% sample were higher due to a high salt concentration. Meanwhile, the total acidity in sample 0% was very high. The moisture, salt concentration and acidity for all samples were significant different (p-value < 0.05). Depending on sensory test, the 0% sample and 2.5% sample did not have good texture and color. Thus, fermented white radish with 5% of salt was better than the others. Comparing with the results done by Joshi and Sharma (2009), the pH of radish fermented was lower. In their study, radish fermented with 2.5% of salt for 16-18 days at a temperature of 20 ± 1°C. The result indicated that the fermented radish had pH 3.64 and acidity 1.80%.

*3.9 Physicochemical parameters in fermented white radish with size variation*

Table 4. showed that for these three samples of fermented white radish indicated that the moisture content, salt concentration and total acidity were significant different (p-value < 0.05). Depending on the test result showed that sample size 1/2 had better texture than the other samples. In that case, fermented white radish with size 1/2 was a better one among three sizes as it gave crisp texture and white color. According to early kimchi fermentation, the pH was from 5.64 to 4.27 and acidity was from 0.48% to 0.89%. Their study showed that the acidity was much higher than this study (Montet *et al*., 2014).

Table 5. Physicochemical analysis in fermented white radish with size variation

| Size variation | pH value | Moisture (%) | Salt (%) | Acidity (%) |
|---|---|---|---|---|
| Size 1 | $4.19 \pm 0.01^{a}$ | $90.04 \pm 0.04^{a}$ | $4.27 \pm 0.05^{a}$ | $0.14 \pm 0.05^{a}$ |
| Size 1/2 | $4.26 \pm 0.00^{b}$ | $88.87 \pm 0.14^{b}$ | $4.50 \pm 0.00^{b}$ | $0.17 \pm 0.00^{b}$ |
| Size 1/4 | $4.19 \pm 0.00^{a}$ | $87.94 \pm 0.14^{c}$ | $4.40 \pm 0.00^{c}$ | $0.19 \pm 0.00^{c}$ |

Different letter in column, the sample are significant different (p-value < 0.05),

Same letter in column, the sample are not significant different (p-value > 0.05),

*3.10 Physicochemical parameters in fermented white radish with and without drying*

Table 6. Physicochemical analysis in fermented white radish with and without drying of white radish

| Fresh and dried | pH value | Moisture (%) | Salt (%) | Acidity (%) |
|---|---|---|---|---|
| F | 4.14 ± 0.00 | 84.13 ± 0.03 | 4.60 ± 0.00 | 0.177 ± 0.00 |
| D | 4.12 ± 0.00 | 85.46 ± 0.03 | 4.70 ± 0.00 | 0.184 ± 0.00 |

D: with drying white radish, F: without drying white radish

Table 6 showed that the results of pH, moisture content, salt concentration and total acidity were not much different between with and without drying of white radish. However, the dried sample had better texture and color than the non-dried sample. Overall, dried white radish fermentation in rice bran bed was the best consideration. The comparison with sinki (a sour pickle from radish rood that was sun dried for two days and fermented 12 days) done by Monet *et al*. (2014). After fermentation, the pH dropped from 6.7 to 3.3 and the moisture was 21%.

*3.11 LAB variation during fermentation with salt variation*

In Fig. 6, the small amount of LAB at the first day of fermentation was observed and LAB count was the same for all samples. The significant increase in LAB of sample 0%, 2.5% and 5% was about $5.10^{3}$CFU/g at the first day. Moreover, the amounts of LAB grown moderately till the third day about $6.10^{13}$ CFU/g because the carbohydrate and less salt concentration led to produce more lactic acid bacteria. Meanwhile, the number of LAB of sample 7.5% and 10% grown slightly during 3 days because of high salt concentration. Comparing with the result done by Doi *et al.* (2013), the numbers of colonies in fermented rice bran was $4.1.10^{6}$ CFU/ml.

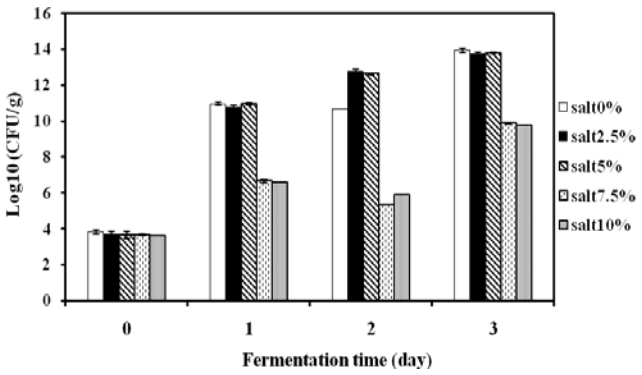



Fig. 6. Result of LAB count in fermented rice bran from day 0 to day 3 with salt variation

*3.11 Lactic acid concentration by HPLC*

Table 7 indicated that the lactic acid in fermented white radish was 10,100 mg/l and in fermented rice bran was 11,250 mg/g of the sample 0% (no salt added). However, the amount of lactic acid in fermented white radish was 10,600 mg/l and in fermented rice bran was 7,765 mg/l of samples 2.5%. Meanwhile, the amount of lactic acid of sample 5% in fermented white radish was 5,626 mg/l and in fermented rice bran was 6,212 mg/l. The amount of lactic acid in fermented white radish was slightly smaller than in fermented rice bran because of suitable salt concentration of sample 5%. The amount of lactic acid of sample 7.5% in fermented white radish was 3,082 mg/l and in fermented rice bran was 1,450

mg/l. There was no lactic acid in the sample that too high salt was added (sample 10%).

Table 7. Lactic acid in fermented white radish and rice bran with salt variation at the last day of fermentation by HPLC

| Salt concentration (%) | Lactic acid (mg/l) | |
|---|---|---|
| | Fermented white radish | Fermented rice bran |
| 0 | 10106.28 | 11256.51 |
| 2.5 | 10619.23 | 7765.90 |
| 5 | 5626.25 | 6212.19 |
| 7.5 | 3082.39 | 1450.78 |
| 10 | 0 | 0 |

The salt concentration was increased, whereas the lactic acid showed a decrease. Acetic acid was also measured in this experiment; however, it was not detected during fermentation. Therefore, this fermentation was homo-fermentation because the LAB converts fermentable sugars to only lactic acid. According to Jing *et al.* (2014), the result of lactic acid in radish brines alter a two week fermentation of red radish was about 6.23g/l. Their result was similar to this study of sample 5% in fermented rice bran.

## CONCLUSIONS

In conclusion, this research showed that the solid-state fermentation of white radish with rice bran gave a pH value of 4.08-4.26, moisture content of 85.00%-88.87%, a salt concentration of 4.5%-4.7% and a total acidity of 0.17%-0.19% in fermented white radish. In addition, the optimal conditions during white radish fermentation were: ratio of rice bran and water (1:1), salt concentration 5%, size 1/2, and drying under the full sun light for duration of two hours. This fermentation was homo-fermentation because LAB converted fermentable sugars to produce only lactic acid. By comparing with Japanese product, it showed that the color, texture, flavor and aroma still not acceptable by Cambodian people. This might be due to different rice bran that was used and shorter fermentation time of rice bran bed.

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