

Lactic Acid Fermentation of Radish and Cucumber in Rice Bran Bed

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Summary

The *Lactobacillus plantarum* (*L. plantarum*) strain XK1.4 isolated from pickled vegetables was applied for cucumber and radish fermentation using rice bran. Fermented radish and cucumber pickles are the lactic acid fermentation products formed through the influence of microorganisms present in the environment. The main objective of the study is to select the appropriate rice bran type (white/yellow rice bran) and treatment methods (roasting time), and also choose suitable fermentation conditions (initial microbial population and added salt content) for traditional pickled cucumber and white radish with appropriate lactic acid content and high acceptability by consumers. The results showed that the quality of white bran was better than yellow bran and less oxidized, the total free fatty acid was also much lower than that of yellow bran. It was found that the lactic acid content analyses provided significant different results for the samples, compared to the control (without inoculant addition). The pickled samples for which *L. plantarum* strain XK 1.4 was used displayed a better fermentation process. The lower concentration of bacteria added in the initial stage, the lower the acid content of the rice bran medium and the fermented products where *L. plantarum* strains were added. *L. plantarum* grew rapidly in rice bran fermenting bed of 10^3 CFU g^{-1} at 25-26 °C and 3% NaCl. With the appropriate selection of fermentation parameters, it only takes about 4 days for the fermentation process (2 days of preparing rice bran medium and 2 days of fermenting white radish and cucumber) with high lactic acid content and consumer's acceptability.

Key words

rice bran, *Lactobacillus plantarum*, salt, pickled radish and cucumber, lactic acid

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Introduction

Pickling is the process of preserving or extending the shelf life of vegetables by either anaerobic fermentation in brine or immersion in vinegar. Lactic acid fermentation increases the shelf life of vegetables and also enhances several beneficial properties, including nutritive value and flavors, and reduces toxicity (Swain et al., 2014). Due to the presence of certain lactic acid bacteria, fermented vegetables can be used as a potential source of probiotics (Swain et al., 2014), such as *L. plantarum*, *L. casei*, *L. acidophilus*, and *Streptococcus lactis* (Tamang, 2009). The properties of lactic acid bacteria isolated from fermented vegetables have shown that their consumption offers many health benefits, increasing the health of gut microbiome and digestive system and enhancing the immune system.

Vegetables are easily perishable commodities due to their high water activity and nutritional values. These conditions are more critical in tropical countries, which favours the growth of spoilage causing microorganisms. In Vietnam, vegetables as cucumber and radish are materials used for pickling, just simply by adding in brine solution and they have a crispy crunchy texture. Fermentation can be accomplished using one of two processes: spontaneous fermentation or inoculation with the use of selected starter cultures. Lactic acid bacteria and yeasts have been reported to be dominant in the fermentation of vegetables. The pH value of fermented vegetables is almost 4.6 or lower, which is sufficient to prevent most bacteria with antimicrobial herbs from being added. Natural fermentation at room temperature, by lactic acid bacteria, produces the required acidity. The acidity or salinity of the solution and the temperature of fermentation determine the flavor of the end product. Besides that, Vietnam is one of largest rice exporters with countless paddy fields from North to South. Rice kernels are composed of about 20% rice husk, 11% rice bran, and 69% starchy endosperm (milled rice) (Dhankhar & Hissar, 2014). Rice bran is a source of protein, fiber and lipid, basically unsaturated fatty acids (Alauddina, 2017). However, rice bran is not consumed as food by humans due to possible hull contamination and its high fiber content. After its production in the mill it undergoes rapid enzymatic hydrolysis caused by lipases activity on the bran lipids released from the cells in the milling process, which results in the decomposition of lipids into free fatty acids. Several researchers suggest that rice bran is also a good medium for vegetable fermentation. *Nukazuke* is a type of Japanese preserved food, made by fermenting vegetables in rice bran, developed in the 17th century. A bed of rice bran is composed of rice bran as a main starting material, mixed with an appropriate amount of water and salt, and preferably with a high-quality starter which is fully fermented by the combination of lactic acid bacteria and yeast fungus, used for pickling vegetables. Nowadays, as the consumer pays a lot of attention to the relation between food and health, the *Lactobacillus* in pickled vegetables may be a beneficial supplement to the intestinal flora (Tamang, 2009). Since pickled vegetables absorb nutrients from the rice bran, they are high in vitamin B1. At higher temperatures, *L. plantarum* dominates producing primarily lactic acid. At an appropriate temperature (around 25 °C), lactic acid bacteria and yeast fungus in addition to enzymes in vegetables ferment sugar (glucose), oils (rice bran oil) and amino acids to produce special flavor which is absorbed in pickled vegetables. There are very few studies regarding the fermentation technology in vegetables using rice bran as a fermented medium;

therefore the objective of this study was to evaluate the quality of the two rice bran varieties, and analyze the quality change of rice bran after roasting at different times in the process as well as storage. Furthermore, effective application of rice bran sources has been selected and processed for the processing of fermented white radish and cucumbers in the rice bran medium.

Materials and methods

Sample Preparation

Rice bran used in this study was collected only from Phuoc Thanh IV Trading-Production company Limited, Vinh Long Province. The industry produces two kinds of rice bran after rice milling (yellow color) and polishing (white color). However, rice bran is not consumed as food by humans due to possible hull contamination, so the samples are checked for their quality and microbiological suitability for human use.

Rice bran batch (10 kg) was roasted by using Roaster 106 (England) which controls the heating temperature of the air (110 °C) at different roasting times (8, 10 and 12 min). After that, processed, heated rice bran was packed in contained clear zipper-top bags. Half of them were stored at 15 ± 1 °C and the other half were stored at 28 ± 2 °C (with the same monitored relative humidity 55%) for 4 weeks.

Vegetables: mature white radish (*Raphanus sativus* var. *Longipinnatus*) and cucumber (*Cucumis sativus*) were harvested in the gardens (Can Tho city). After harvest, the vegetables were washed with sanitary water until the dirt was removed, drained and peeled (for white radish) or unpeeled (for cucumber), ready for the fermentation process. Chinese cabbage, garlic, and chili were washed and chopped.

Starter Culture Preparation: lactic acid bacteria strain XK 1.4 was isolated from cucumber showing the strongest antibacterial activity (Thuy et al., 2017). Results of DNA sequencing showed that sequences of XK 1.4 strain were similar to *L. plantarum* (similarities 97%) (Thuy et al., 2017). After testing the antibacterial activity, XK 1.4 strain was selected and could be implicated in the vegetable fermentation as a starter culture. The culture was maintained at -50 °C in 20% glycerol stocks. For use, *L. plantarum* was proliferated in liquid MRS medium (peptone, meat extract, and yeast extract) in 250 mL flasks. The medium was adjusted to a pH of 6.2 using citric acid and sterilized in an autoclave at 121 °C for 15 min at a pressure of 1.033515 bar. 250mL Erlenmeyer flasks with an effective volume of 150 mL were used and seeded with selected *Lactobacillus* culture. These inoculated media were incubated at 37 °C with shaking (140 rpm) for 24 hours. The achieved initial density of bacteria was 5.0 log cells mL⁻¹.

Experimental Design

The studies were conducted on the basis of surveying the effect of roasting time (8-12 min) at 110 °C for two types of white rice bran (WBR) and yellow bran rice (YBR) on quality and storage capacity.

After selecting the type of rice bran and the appropriate treatment conditions, the rice bran bed was added to inoculated bacteria *L. plantarum* inoculants which were applied at bacterial densities (10^2 , 10^3 , 10^4 CFU g^{-1} of rice bran) and the amount of salt was added from 2 to 4% of the rice bran weight. The control sample was prepared at the same time without inoculant addition. Samples (radish, cucumber) were prepared and carried out in the fermentation according to the following steps:

Step 1. The three essential ingredients used for fermentation are: bran, salt and water. The water needs to be boiled and cooled before added in order to remove the chlorine in the water. Rice bran is roasted. Scraps of vegetables [cabbage outer leaf (3%), garlic (2%) and chili (2%)] are added. These are not for eating but for making the rice bran bed tasty. Those vegetables also can supplement nutrients, antiseptics, antioxidants as well as speed up the fermenting process and moderate moisture.

Step 2. A bed of rice bran is composed of rice bran as the main starting material, mixed with an appropriate amount of water (48% of the rice bran weight) and salt (2-4% of the rice bran weight) as needed to achieve the right consistency. It was mixed thoroughly by hand. This serves as the rice bran bed. At this time, the rice bran bed contains a culture of active single-celled organisms, mostly lactobacilli and yeast. The rice bran fermenting bed is added to inoculated bacteria *L. plantarum* inoculants at different densities (10^2 , 10^3 , 10^4 CFU g^{-1} , as mentioned above) with the control sample without inoculant addition. The surface of the rice bran bed is flattened and a clean paper is used to wipe off the bran on the side of the box (width: 18 cm, height: 14 cm and length: 28 cm). The box is sealed and left at room temperature (25-26 °C) for 2 days (Fig. 1).

Step3. After 2 days, push vegetables completely inside the rice bran bed (white radish is washed, peeled, cut into pieces and the whole fruit is left for cucumber) in a single layer, ensuring there is no overlapping. Cover with the rice bran bed. Lightly press down on the top to ensure everything is packed in (Fig. 2). Allow to sit for 2-3 days in a dry, non-musty place. Be sure to stir the contents with clean hands at least once a day to ensure proper pickling and avoid sourness. Replace the lid and weights after each stir.

Step 4. After 2-3 days, pickled vegetables develop a slightly sour, fermented odor, which means it was starting to produce lactic acid. Then the vegetables are taken out, washed with potable water, consumed within a few days or packed and stored.

Each treatment was performed in 3 replicates. The fermentation was conducted at room temperature (25 to 26 °C). At the end of the fermentation period, the quality of the product was analyzed for lactic acid and sensory values.

Chemical Characteristics Analysis

Lactic acid bacteria total counts: total plate count of lactic acid bacteria was conducted following the method as described by Hadioetomo (1993), 10 mg of fermented rice bran was diluted in 90 mL phosphate buffer and 1ml was then pipetted onto MRS (de Man, Rogosa, and Sharpe)-agar and incubated at 37 °C for 2 days before counting the colony formed.

Determination of the Moisture Content: the moisture content of the rice bran was measured by using standard methods (AOAC, 2000).



Figure 1. Preparation of rice bran fermenting bed



Figure 2. Pickling vegetables (radish and cucumber)

Determination of Total Free Fatty Acid (FFA): the total FFA content was determined according to a titration method (Sharma et al., 2014). Exactly 5 g of rice bran samples (WRB and YRB) and 50 mL benzene were added into a flask for extraction of free fatty acids. After stirring for 30 min, 25 mL extraction liquid was taken and mixed with a 25 mL 0.04% phenolphthalein ethanol solution in the flask. The mixture was titrated with 0.05 M KOH until a light pink colour appeared. The total FFA was calculated using equation 1.

$$\text{FFA (mg KOH g}^{-1}\text{)} = \frac{\Delta V \times 0.05 \times 56.1 \times \left(\frac{50}{25}\right) \times 100}{m \times (100 \times M)} \quad (1)$$

where ΔV is volume of titration (ml) with 0.05 M KOH; 56.1 is KOH mg equivalent; m is weight of rice bran sample (g); M is percentage of rice bran sample moisture (%).

The total FFA (%) content of samples was analysed (%) over a storage time of 0 to 4 weeks, at 1 week interval.

Total Acid (expressed as lactic acid) Measurement: the total acidity was determined by titrating 10 mL of fermenting liquor in 50 mL Erlenmeyer flask using 0.1N NaOH and 1% phenolphthalein as the indicator; the total acidity was expressed as lactic acid (AOAC, 2000).

Nutrients Analysis: the basic nutrients protein, moisture and fat of rice bran were determined by using standard methods (AOAC, 2000). The total carbohydrate content was determined according to the method of McCready (1970) and Dubois et al. (1956). Dietary fibre content was determined by an enzyme-gravimetric method according to Prosky et al. (1988). The method gives the sum of insoluble (IDF) and soluble dietary fibre (SDF) contents.

Sensory Evaluation: the sensory values of these final products were also evaluated by using a 9-point scale. Fifty panelists were trained to evaluate overall acceptance based on quality of the products. Every product was rated for overall liking by each panelist. The evaluation was conducted in a well-ventilated laboratory fitted with fluorescent lights. The temperature in the evaluation room was 25 ± 1 °C.

Statistical Analysis: statistical analysis was carried out using the Statgraphics Centurion XV software, while means were separated by Least Significant Difference (LSD) test at 5% level of significance. The XLSTAT 2007 (StatPoint Technologies Inc., USA) was used for sensory data analysis.

Results and discussion

Treatment and Storage of Rice Bran

The Chemical Compositions of Rice Bran

The analysis results of chemical composition of rice bran were shown in Table 1. They showed that the moisture content of white bran was about 11.7%, lower than that of yellow bran (12.5%). These obtained results were almost similar to the research results of Lien and Thuy (2016) and Hettiarachchy (2009) with the value of white milled rice bran of 11.9% and the defatted bran about 10.5-12.0%, respectively. The carbohydrate content of white bran was about 53.74%, higher than that in yellow bran (49.07%). The obtained result was slightly higher than the result of Hernandez

Table 1. Chemical composition of rice bran

Rice bran type	Moisture content (%)	Carbohydrate content (%)	Protein content (%)	Lipid content (%)	Fiber content (%)
White rice bran	11.70 ^a ± 0.07	53.74 ^b ± 0.74	11.42 ^a ± 0.27	14.39 ^a ± 0.02	4.66 ^a ± 0.05
Yellow rice bran	12.50 ^b ± 0.11	49.07 ^a ± 0.89	11.89 ^b ± 0.11	15.76 ^b ± 0.27	6.56 ^b ± 0.12

Note: Means ± SD with the different letter in the same column are significantly different ($P < 0.05$)

et al. (2000) with a value of 48.3%. In contrast, the protein content of the yellow bran and white bran was about 11.89% and 11.42%, lower than the protein content in the study of Hernandez et al. (2000) with a value of 13.4%. The yellow bran contained about 15.76% lipid, it was almost similar to research results of Cheruvanky (2003) with the proposed fat content in rice bran about 15-20%. The differences in the milling process greatly affect the chemical composition of rice bran (Houston, 1972). Mir et al. (2017) also posted that geographical conditions and rice varieties also significantly affected the quality of bran. Over time, the bran can be oxidized due to lipase in the bran, so heat treatment is also the way that effectively inhibits the activity of this enzyme. Besides that, the white bran, because of its high sugar and low lipid content was chosen to prepare a pickling bed for vegetable fermentation.

Effect of Roasting Time on Rice Bran Stability

After production in the mill, rice bran undergoes enzymatic hydrolysis caused by lipases activity on the bran lipids released from the cells in the milling process, which results in the decomposition of lipids into free fatty acids (FFA). Due to its nutritional value, the low cost and potential use in human nutrition, several studies have been conducted to evaluate its use in food (Issara and Rawdkuen, 2016). The heat treatment process on rice bran had a good influence on the quality of the bran. The rice bran roasted at a short time (8 min) showed a significantly higher increase in FFA content for 4 weeks. However, if the bran is subject to a thermal treatment of high temperature and proper time (10 to 12 min) immediately after milling, the lipase activity is reduced, thus producing bran with a longer shelf life, which is suitable for human consumption. A comparative study of free fatty acid of two kinds of rice bran at different treatment times was realized in Fig. 3.

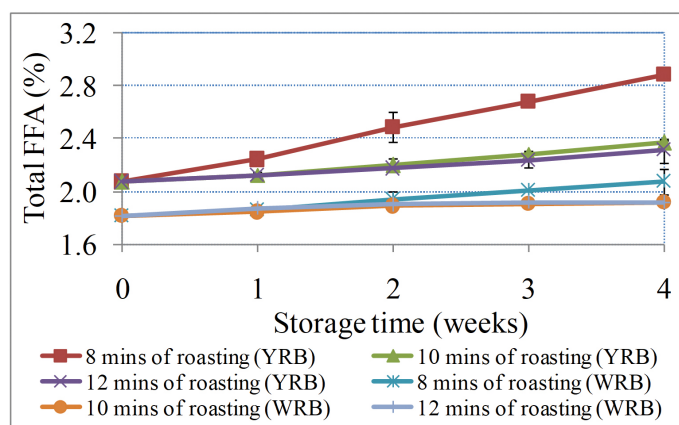


Figure 3. Changes of FFA (%) of rice bran during storage (YRB: Yellow rice bran, WRB: White rice bran)

Thermal treatments to bran rice for stabilization have been applied, such as microwaving (Patil et al., 2016), extruding (Kim et al., 2006), steaming (Juliano, 1985), dry heat stabilization (Shi-Wen et al., 2018) and ohmic heating (Dhingra et al., 2012). Storage significantly affected the lipid oxidation in rice bran. The obtained results indicated that the storage temperature showed a significant difference in the content of FFA in the stored bran samples, in both yellow and white rice bran ones, and at both storing conditions (Fig. 4).

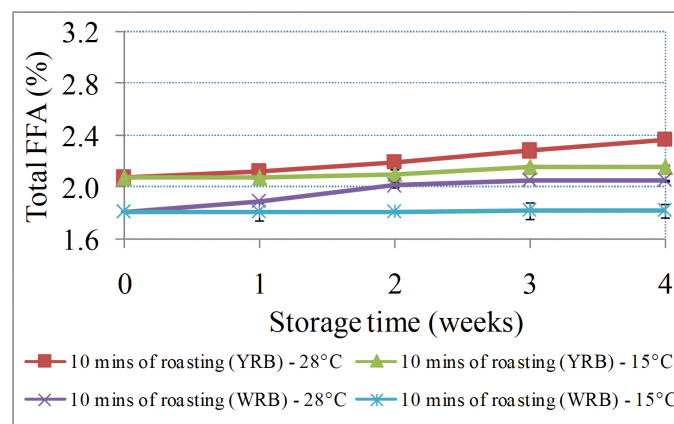


Figure 4. Changes of FFA (%) of rice bran at different roasting and storage temperatures (YRB: Yellow rice bran, WRB: White rice bran)

It was observed that the total FFA in yellow and white rice bran increased 1.13 to 1.14 times over a 4-week period from the initial value of 2.07% and 1.81%, respectively, stored at 28 °C. However, at low temperature storage (15 °C), the total FFA of roasted rice bran increased at a slower rate (for yellow rice bran) or did not seem to change (for white rice bran) after 4 weeks of storage. The quality of white bran was better than that of yellow bran and less oxidized, the total FFA was also much lower than that of yellow bran. Therefore, white bran is used for subsequent research activities.

Fermentation of Vegetables

Cell Growth of *L. plantarum* under Different Initial Bacterial Density

Rice bran fermenting bed is used for pickled vegetables to give products a salty and sour taste and a special flavor. For traditional fermentation, the rice bran bed is initially prepared by natural fermentation of vegetables in rice bran until a fermenting culture has been established. The starter culture is added to the vegetables prior to fermentation for accelerating the process and for the dominance of a particular microflora on the raw material

to prevent pathogen contamination further (Holzapfel, 2002). However, according to Ono et al. (2014), microbial communities in all tested nukadoko samples were dominated by *L. plantarum*. In this study, *L. plantarum* was used as a starter culture to promote the initial fermentation of radish and cucumber. Besides that, *L. plantarum* could survive longer in the increasingly acidic pickled vegetable environment and it was detected as a predominant species in naturally fermented nukadoko samples (Sakamoto et al., 2010). The results of *L. plantarum* propagation in rice bran fermenting bed can be observed in Fig. 5. The time required for reaching the maximum values of cell biomass increased from 12 to 48 h in the rice bran bed. After 48 hours of fermentation, at high initial densities of 10^3 and 10^4 CFU g^{-1} , the cell had grown to 3.6 log CFU g^{-1} and 4.5 log CFU g^{-1} , respectively; while at the lower initial concentration (10^2 CFU g^{-1}), the cell achieved only 2.9 log CFU g^{-1} , and the lowest concentration of 1 log CFU g^{-1} with the control sample.

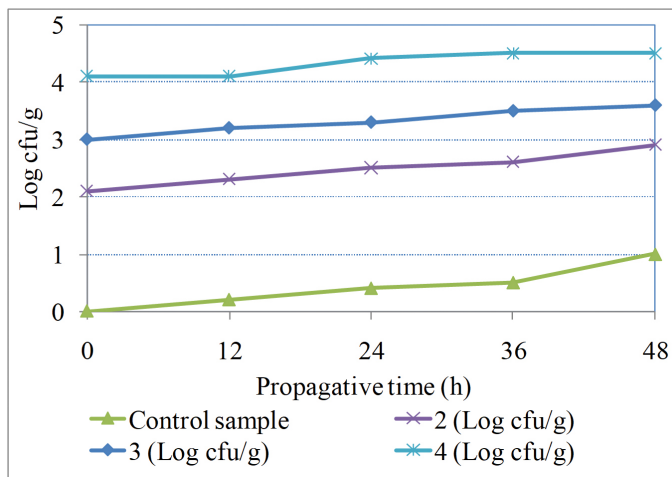
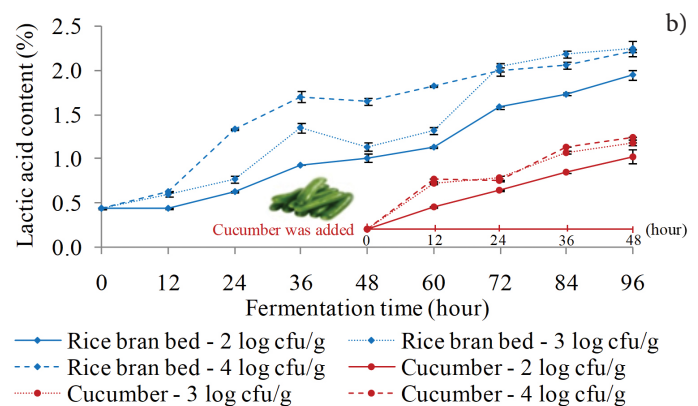
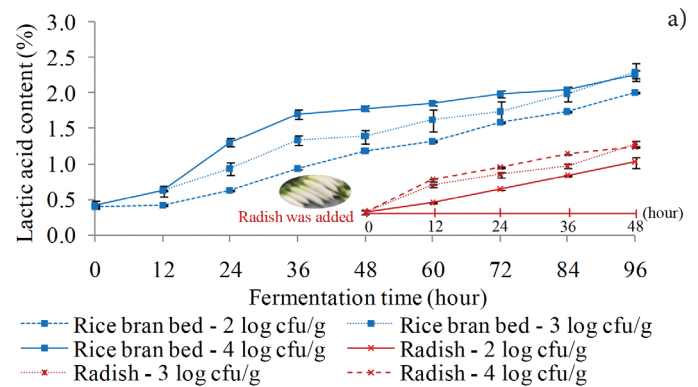


Figure 5. Growth of *L. plantarum* in rice bran bed (log CFU g^{-1}) during propagative time at different initial density

Effect of *Lactobacillus plantarum* in Fermentation

In a pickling bed of fermented rice bran, *L. plantarum* started growing during the first 48 hours and consumed sugars. The amount of starter culture had a significant influence on the final acid content, the sensory quality of radish and cucumber including the texture, taste and odor. The higher sourness and acidic odor were observed in the pickled vegetables with higher addition of starter culture. During the growth and multiplication of *L. plantarum* in the first 48 hours, lactic acid significant increases were recorded in comparison to the initial state (1.1 to 1.8% lactic acid) with the added concentration of *L. plantarum* 10^3 to 10^4 CFU g^{-1} , respectively, causing a further lactic acid increase in pickled vegetable in the next 48 hours. Over the next 48 hours, white radish and cucumber fermentation progressed. The lactic acid content of white radish and cucumber reached 1.234 to 1.284% and 1.176 to 1.234%, respectively at the concentrations of 10^3 and 10^4 CFU g^{-1} *L. plantarum* were added. The lower lactic acid contents were measured in fermented samples (1.025%) at 10^2 CFU g^{-1} of starter culture (Fig. 6).



Note:

Rice bran bed – 2 log CFU g^{-1} : initial *L. plantarum* concentration of 10^2 CFU g^{-1} was added to the bran.

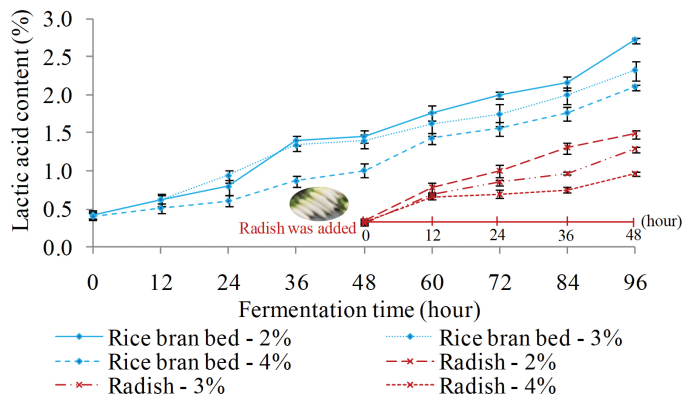
White radish – 3 log CFU g^{-1} : white radish fermented in rice bran was added 10^3 CFU g^{-1} *L. plantarum*

Cucumber – 3 log CFU g^{-1} : cucumber fermented in rice bran was added 10^3 CFU g^{-1} *L. plantarum*

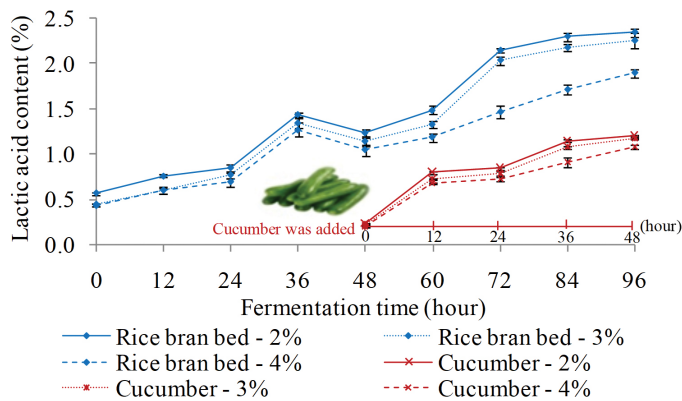
Figure 6. Lactic acid production in white radish (a) and cucumber (b) at different *L. plantarum* concentration

Effect of Salt

Brining is also an important step in vegetable fermentation. Dry salt is added to vegetables for pickling. The lactic acid contents achieved were higher when salt levels were used 2 to 3% and lower when 4% of salt was used (Fig. 7). The addition of salt is necessary to increase the osmotic pressure which allows water and sugars to be pulled from the cabbage cell walls, which are used as nutrients by *L. plantarum*, to prevent spoilage and pathogenic microorganisms from growing (Doyle and Glass, 2010) and prevent texture softening as a result of the decrease in endogenous pectolytic enzyme activity, naturally found in vegetable. In comparison, high salt concentration delayed the maturation of pickled product and inhibited the metabolism of lactic acid bacteria (Xiong et al., 2016). Moreover, a high level of dietary sodium is associated with a high prevalence of hypertension, prehypertension and, possibly, other adverse effects on health. Many national and international health organizations recommend that sodium intake be significantly decreased (Doyle and Glass, 2010). Therefore, consumers today prefer low sodium foods. Attempts to reduce sodium content in food processing may be needed to create tasty products and to meet consumer requirements for disease prevention.



a) Radish



b) Cucumber

Note:

Rice bran bed – 2%: 2% salt was added in the bran

White radish – 3%: White radish fermented in rice bran bed added 3% salt

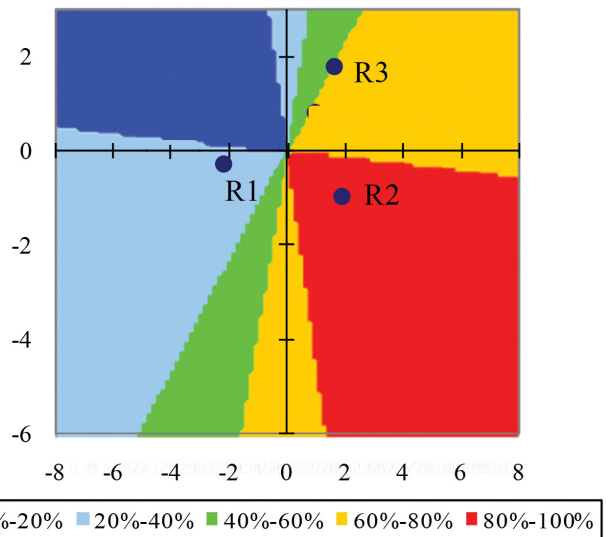
Cucumber – 3%: Cucumber fermented in rice bran bed added 3%

Figure 7. Effect of salt concentration on lactic acid content in rice bran bed and vegetables (radish and cucumber)

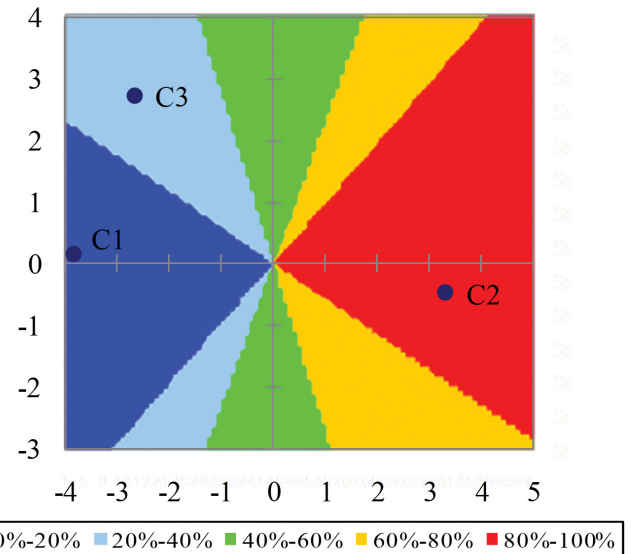
Now, many studies about the effect of salt on acid production during vegetables have been reported. Cabbage, for example, is salted with 2-3% sodium chloride (NaCl) (Perez-Díaz et al., 2013; Holzappel, 2002). The typical salt concentrations added to cabbage for sauerkraut fermentation could range between 2 to 10% (w/w) (Xiong et al., 2016).

Lactic acid bacteria can tolerate high salt concentrations. This salt tolerance gives them an advantage over less tolerant species and allows lactic acid fermentation that inhibits the growth of non-desirable organisms (Rao et al., 2004). The salt levels of 2.5–3.0% in kimchi exhibited antimutagenic and anticancer effects (Park et al., 2014). In general, lactic acid bacteria found in the raw materials act as a starter culture during vegetable fermentation. Vegetables are found to be an excellent source of carbohydrates that are converted into lactic acid. During vegetable fermentation, the 2-5% brine solutions are added to provide hypertonic conditions that inhibit the formation of spoilage-causing organisms during and after the fermentation process.

Samples of pickled cucumber and radish were evaluated by preference score (Fig. 8). The results showed that the most acceptable pickled products by consumers were samples fermented in rice bran bed with 3% salt added (C2 and R2).



a) Radish



b) Cucumber

Note:

R1, R2, R3: Radish fermented in rice bran bed added 2%, 3%, 4% salt, respectively

C1, C2, C3: Cucumber fermented in rice bran bed added 2%, 3%, 4% salt, respectively

Figure 8. Preference mapping of pickled products

These samples were evaluated with bright color, good taste and aroma. Meanwhile, the samples coded C1, C3 and R1 had low acceptability of consumers (20-40%). The results from preference mapping could confirm that the product has a high acceptable value and appropriate lactic acid content (1.1-1.3%) when the raw material (radish and cucumber) was fermented in the rice bran bed with 48% water, 3% salt added and at 10^3 CFU g^{-1} of starter culture. From the above investigation, a product of Vietnamese vegetables (cucumber and radish) pickled in a salted rice bran bed is rich in bright color, delicious flavor, crunchy structure and high nutritional values.

Conclusion

White rice bran with high carbohydrate content and low lipid content was suitable as a culture for lactic acid fermentation. Results showed that hydrolytic rancidity of rice bran could be prevented by roasting for 10 min (at 110 °C) and that the recommended storage condition for roasted rice bran was 15 °C in zipper-top bag in 4 weeks. With an initial density of 10^3 CFU g^{-1} , lactic acid bacteria grew rapidly in the rice bran bed (obtaining 3.6 log CFU g^{-1} after 48 hours) and the content of lactic acid also increased significantly during fermentation (white radish 1.234 to 1.284% and cucumber 1.176 to 1.234, respectively after 96 hours). A salt concentration of 3% was suitable for lactic acid fermentation and the obtained product had the highest acceptance by consumers. The above results could provide a theoretical basis for the actual treatment and storage of yellow and white rice bran. Besides, producing a delicious and reliable product using *L. plantarum* strain XK 1.4 in pickled radish and cucumber was believed to significantly contribute to the food industry.

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