



ORIGINAL ARTICLE

Kinetic Analysis of Cell Growth and Acid Formation in Radish (*Raphanus sativus L.*) Pickle

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**ABSTRACT**

This work was conducted to investigate the kinetics of Lactic Acid Bacteria growth and lactic acid production during 16 days of radish pickle fermentation. Pickle was prepared following the traditional process. Parameters like lactic acid bacteria count, titratable acidity (% lactic acid) and pH were determined. The fermentation was carried out in anaerobic condition using air tight jars. The samples were kept in three different temperatures for fermentation namely; 4°C, 37°C and 30 ± 3°C. No significant differences (p>0.05) in acidity and pH were observed among samples after the first and second days of fermentation. Acidity and pH of pickle observed from day three through the end of the fermentation period were found to be significantly different (p<0.05). Pickle acidity increased and pH decreased during fermentation at refrigerated, room and incubation temperatures. The number of LAB increased significantly during fermentation, starting at 4.56 log<sub>10</sub> CFU/g and reaching 6.4 log<sub>10</sub> CFU/g at refrigerated temperature (T<sub>1</sub>), 10.45 log<sub>10</sub> CFU/g and 10.2 log<sub>10</sub> CFU/g at room temperature (T<sub>3</sub>) and incubation temperature (T<sub>2</sub>) respectively.

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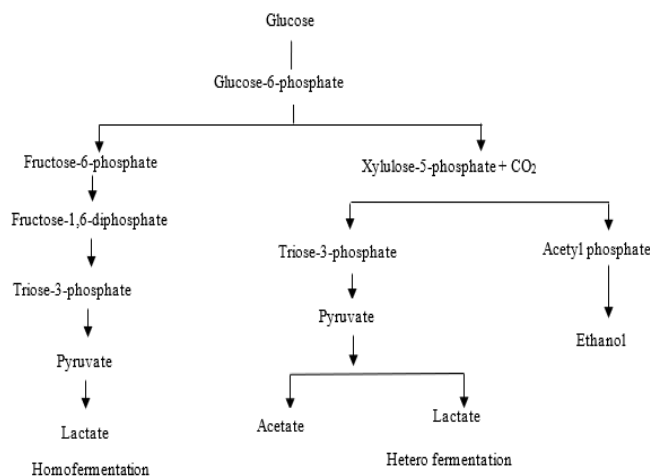
## 1. INTRODUCTION

Fruits and vegetables are rich in water-soluble vitamins, provitamin A, phytosterols, dietary fiber, minerals, and phytochemicals, consumption of which can help to prevent chronic conditions such as high blood pressure (Cagno et al., 2013). Fermentation is a widely utilized process that produces new products with unique flavors, nutritional profiles and distinct physicochemical properties (Saranraj et al., 2017). Fermentation relies on uses of microbial activity to produce metabolites that can suppress unwanted microflora in foods (Paul Ross et al., 2002). Fermentation is an efficient, low-energy method for preserving food, extending shelf life, and reducing reliance on refrigeration, makes suitable for rural and developing regions with limited access to advanced technology (Saranraj et al., 2017).

Pickling is a lactic acid fermentation process which preserves fruits and vegetables by fermenting and salting ingredients to inhibit microorganisms (Joshi & Sharma, 2009). In pickling process, jars are filled and kept in sun that the heat of sun reduces the presence of bacteria and mold and contribute to preservation of pickle (Davison, 2018). In South Asian country like Nepal, India, Pakistan, Srilanka different varieties of pickle are prepared and consumed (Davidson, 2014; Davison, 2018). In Pakistan and Srilanka, pickle is known as *achaar* and *achecharu* respectively. *Achcharu*, typically prepared from carrots, chili powder, shallots, and ground dates mixed with vinegar, garlic, crushed ginger, green chilies, and mustard seeds, and matured in a clay pot. Indian pickle (*Achaar*) is often prepared in three ways (using salt/brine, oil, and vinegar) and can include a variety of fruits (Anon, 2024). Certain unwanted substances such as tannins, polyphenols, and phytates that might be present in raw foods are destroyed or detoxified with the help of fermentation (Das & Deka, 2012).

Species such as *Leuconostoc mesenteroides*, *Lactobacillus brevis*, *Lactobacillus plantarum*, *Pediococcus cerevisiae*, *Streptococcus thermophilus*, *Streptococcus lactis*, *Lactobacillus citrovorum*, *Lactobacillus bulgaricus*, *Lactobacillus acidophilus*, *Bifidobacterium bifidus*, and related lactic acid bacteria are representative organisms involved in lactic acid fermentation (Steinkraus, 2002). Lactic acid bacteria like *Lactobacillus*, *Leuconostoc*, and *Pediococcus* naturally present on fruits and vegetables can spontaneously ferment them. Lactic acid bacteria (LAB) are crucial because they rapidly acidify the raw material during vegetable fermentation. They improve taste, flavor, and texture of fermented foods (Abedi & Hashemi, 2020). LAB produce organic acids (like lactic acid), bacteriocins, ethanol, aroma compounds, exopolysaccharides (EPS), and various enzymes that inhibit the growth of spoilage and pathogenic bacteria (Yan & Tiejin, 2017). LAB produces two types of EPS such as homopolysaccharides and heteropolysaccharides. The majority of LAB that produce EPS are members of the *Streptococcus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, and

*Lactobacillus* genera (Prajapati & Patel, 2013). Amylase, phytase, phosphatase, and other LAB enzymes that have been isolated from fermented vegetables have been observed to exhibit enzymatic activity (Swain & Ray, 2017). LA is formed from hexoses and pentoses under anaerobic conditions by glycolysis pathway and is shown in **Figure 1**.



Source: (Abedi & Hashemi, 2020)

**Fig 1.** Glycolysis pathway

Using probiotic starter cultures like *Lactobacter plantarum*, *Lactobacter rhamnosus*, *Lactobacter gasseri*, and *Lactobacter acidophilus* guarantees consistent and reliable performance (Montet et al., 2014).

Lactic acid bacteria (LAB) are microorganisms that primarily convert sugars into lactic acid through fermentation. Within this bacterial group, there are two subgroups: homo-fermentative and hetero-fermentative. Homo-fermentative bacteria, genera include *Lactococcus*, *Streptococcus*, and *Pediococcus* generate a single fermentation product, or LA. Hetero-fermentative bacteria, genera *Lactobacillus* and *Leuconostoc* generate LA along with significant amount of ethanol, acetate and CO<sub>2</sub> through 6-phosphogluconate/phosphoketose pathway (Steinkraus, 2002).

Radish (*Raphanus sativus L.*) is a widely cultivated root vegetable with an enlarged taproot, rich in nutrients such as carbohydrates, protein, crude fiber, and vitamin C as shown in **Table 1** (Yousaf et al., 2021). In addition to its high nutritional content, radish has medicinal potential for the treatment of ulcers, hepatic inflammation, urinary tract infections, heart problems, and stomach ailments (Liu et al., 2022). This study aimed to investigate cell growth kinetics, pH variation, and acid production during pickle fermentation under anaerobic conditions at three temperatures: refrigerated (4°C), incubation (37°C) and room temperature (30±3°C) to optimize recipe and processing parameters.

**Table 1:** Nutritional composition of Raddish (*Raphanus sativus* L.) per 100 g fresh weight

Parameter	Value
Protein (mg)	570
Fat (mg)	70
Carbohydrate (mg)	3030
Fiber(mg)	320
Potassium(mg)	380
Calcium (mg)	148
Vitamin C(mg)	38.8

Source: : (Gamba et al., 2021)

## 2. MATERIALS AND METHODS

### 2.1 Materials

Radish (*Raphanus sativus*), was collected from the local market of Dharan. Forty-five food grade, air tight, odorless and non-breakable Polyethylene Terephthalate (PET) jars (250 mL) were used as fermentation container.

### 2.2 Methods

#### 2.2.1 Recipe for pickle

The recipe for pickle was obtained from face-to-face survey method. Respondents were students of Central Campus of Technology, local people of Dharan-14 area and local people of Ratuwamai-5 Municipality. Respondents of central campus of technology were selected in such a way that maximum possible districts of Nepal could be represented. Forty respondents were asked about 5-6 questions regarding the pickle recipe and the most common recipe shown in **Table 2** was selected for the preparation of pickle.

**Table 2.** Recipe for pickle

Ingredients	Quantity (per kg of Radish)
Salt	50-60g
Turmeric	8-10g
Mustard oil	145mL
Chili (Red)	200g
Mustard powder	20g

#### 2.2.1 Preparation of radish pickle

Radish pickle was prepared by the procedure given by Chakraborty & Roy, (2018) with a slight modification. Fresh Radish (*Raphanus sativus*) was collected from local market and preliminary treatments were done such as cleaning and washing with potable water. Radishes were cut into uniform pieces (4cm×0.5cm×0.5cm) and sun-dried for 6 h. Wilted radishes were mixed with oil, chili, mustard powder, salt, turmeric powder and filled into 250 mL PET jars. Jars were capped tightly and kept

for fermentation at refrigerated temperature ( $T_1, 4^\circ\text{C}$ ), incubation temperature ( $T_2, 37^\circ\text{C}$ ) and room temperature ( $T_3, 30\pm 3^\circ\text{C}$ ) for 16 days.

### 2.3 Analytical procedure

Fermented pickle was taken daily up to day 16 for analysis on different parameters.

#### 2.3.1 Determination of pH

pH of pickle were measured directly with a calibrated digital pH meter (AOAC, 2005). Briefly, 5 g of pickle were crushed and combined with 5 mL of pickle brine the resulting mixture slurry ensures that the pH reading represents the combined sample rather than just the liquid or solid portion. Following the pH measurement, the same sample was used to determine the pickle's total acid content (% lactic acid) via titration, as described in the literature (Wu et al., 2023).

#### 2.3.2 Determination of acidity

Titrateable acidity was measured as described in AOAC, (2005). It was expressed as a percentage of lactic acid and percentage lactic acid was converted to gram per liter.

#### 2.3.3 Microbial analysis of Lactic Acid Bacteria

The acid-producing bacteria were counted using a colony counter as described by Ghimire et al., (2020). . Briefly, 10 g of sample was homogenized with 90 mL of 0.85% (w/v) sterile saline, blended for 5 minutes, and serially diluted up to  $10^8$  CFU/mL. Aliquots were plated on Lactobacillus MRS Agar using the spread plate technique and incubated anaerobically at  $30^\circ\text{C}$  for 48–72 h and colonies were counted.

### 2.4 Statistical analysis

Triplicate experimental data were analyzed for ANOVA using IBM SPSS Statistics version 27. Means were compared by Tukey's HSD test at a 5% significance level.

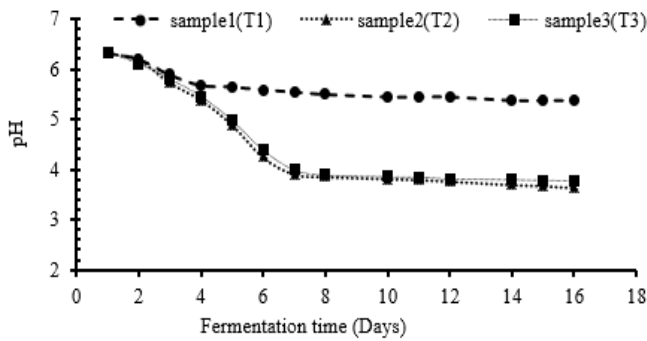
## 3. RESULTS AND DISCUSSION

Radish pickle was prepared, and its percentage acidity, pH, and microbial load were analyzed as outlined in the materials and methods section.

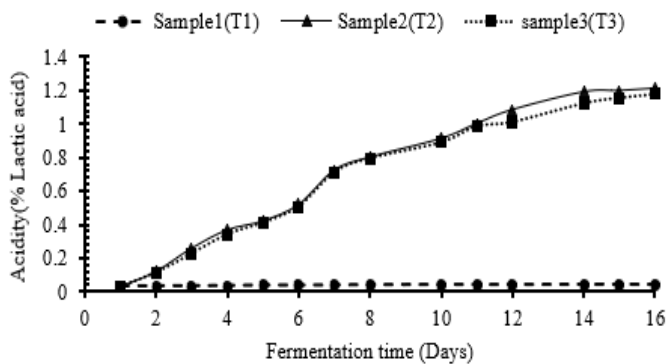
### 3.1 Titratable Acidity (TA) and pH

Titrateable acidity and pH are regarded as the main quality characteristics of pickle for its sour and flavor. Acid concentration and pH value are changed continuously during the pickle fermentation period (Wu et al., 2023). The sour taste in fermented foods mainly results from carbohydrates being converted into organic acids like lactic and acetic acids during fermentation, which lowers the pH (Swain & Ray, 2017). The

fermenting ‘radish pickle’ remained in three different temperatures. Refrigeration temperature ( $T_1$ ), Incubation temperature ( $T_2$ ), Room temperature ( $T_3$ ) respectively. The change in pH during the fermentation period depicted in **Figure 2**. pH of *radish pickle* slightly reduced from 6.32-5.38 in refrigerated temperature, decreased significantly ( $p < 0.05$ ) from 6.32-3.64 in incubation temperature, 6.32-3.78 in room temperature over 16 days fermentation period. Our findings were similar to the values reported by Lu et al., (2001) who observed similar pattern of pH change during the fermentation of cucumber juice. Jung & Jeon, (2007) explained that the pH decrease was due to fermentation temperatures. Ji et al., (2009) and Tamang & Tamang, (2010) suggested that fall in pH over the fermentation time is due to continual microbial growth where the fermentable sugars get converted into lactic acid. In this study, the pH of the pickle decreased sharply during the first 8 days, followed by a slower rate of decrease until 16 days of fermentation, which is similar with the studies of Kang et al., (2004). According to Yan & Tiejin, (2017), throughout fermentation, a gradual increase in fermenting bacteria produces organic acids, leading to a decrease in pH.



**Fig. 2.** Changes in pH during the natural fermentation of *radish pickle* in three temperatures

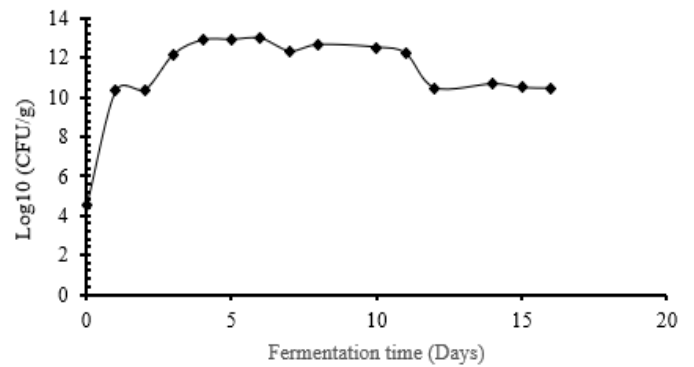


**Fig. 3.** Changes in acidity during the natural fermentation of *radish pickle* in three temperatures.

**Figure 3** illustrates the changes in titratable acidity during natural fermentation of radish pickle. A significant increase in acidity as lactic acid ( $p < 0.05$ ) was observed, rising from 0.03% to 0.042% at refrigerated temperature, 0.03% to 1.21% at incubation temperature, and 0.03% to 1.178% at room temperature. These findings align with Ghimire et al., (2020), who reported that acidity increases and pH decreases as fermentation progresses.

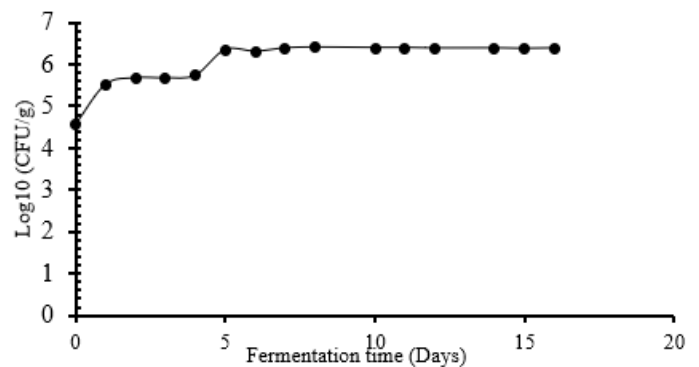
### 3.2 Lactic acid bacteria development

**Figure 4** illustrates the LAB population dynamics during natural fermentation of radish pickle at room temperature ( $T_3$ ). The initial LAB count was 4.56 log<sub>10</sub> CFU/g, which increased significantly by day 3 ( $p < 0.05$ ), and peaked at 12.96 log<sub>10</sub> CFU/g on day 6, followed by a decline through day 16.



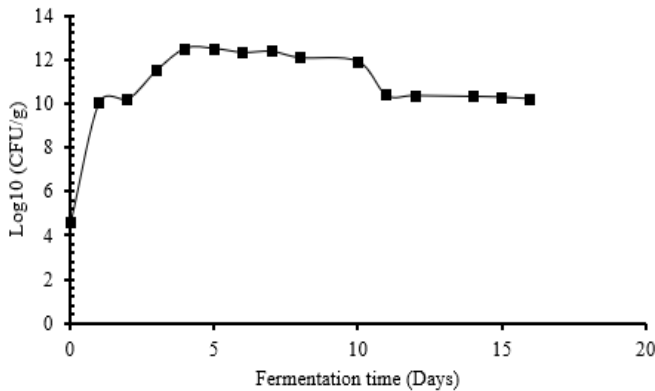
**Fig. 4.** Changes in LAB population during the natural fermentation of *radish pickle* in room temperature ( $T_3$ )

**Figure 5** depicts LAB population dynamics in radish pickle fermentation under incubation temperature ( $T_2$ ). Starting at 4.56 log<sub>10</sub>CFU/g, LAB counts increased steadily to 11.49 log<sub>10</sub> CFU/g by day 3, then declined to match room temperature levels.



**Fig. 5.** Changes in LAB population during the natural fermentation of *radish pickle* in incubation temperature ( $T_2$ )

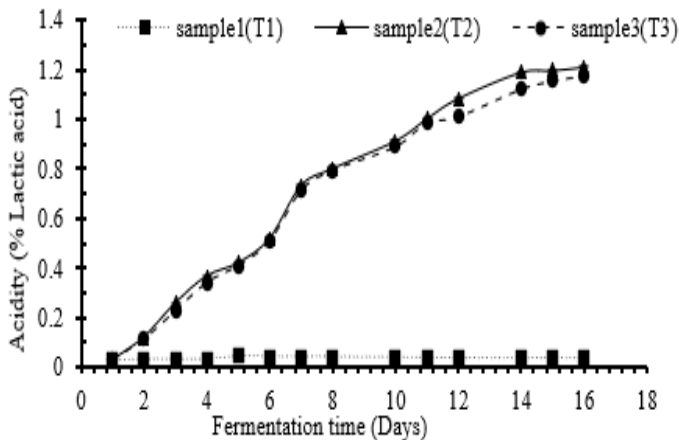
**Figure 6** illustrates the LAB population changes during natural fermentation of radish pickle at refrigerated temperature (T<sub>1</sub>). LAB counts rose slightly to 5.67 log<sub>10</sub> CFU/g by day 3, increased to 6.4 log<sub>10</sub> CFU/g by day 8, and then stabilized at this level for 16-day fermentation. The initial exponential LAB growth likely resulted from rapid heterofermentative rod and homofermentative tetrad development. Subsequent population stability (days 3-5) might be influenced by factors such as the potential absence of *L. cellobiosus* and dominant growth of *L. plantarum* and other homolactics, as observed in other studies (Karki et al., 1983). Yeast/mold inhibition occurred via LAB metabolites disrupting membrane potential, active transport, intracellular pH, and metabolic functions (Savard et al., 2002).



**Fig. 6.** Changes in LAB population during the natural fermentation of radish pickle in refrigerated temperature (T<sub>1</sub>)

### 3.2.1 Effect of Fermentation time and Temperature on acidity

As illustrated in **Figure 7**, radish pickle stored at incubation temperature exhibited higher acidity than those stored at room or refrigerated temperatures.

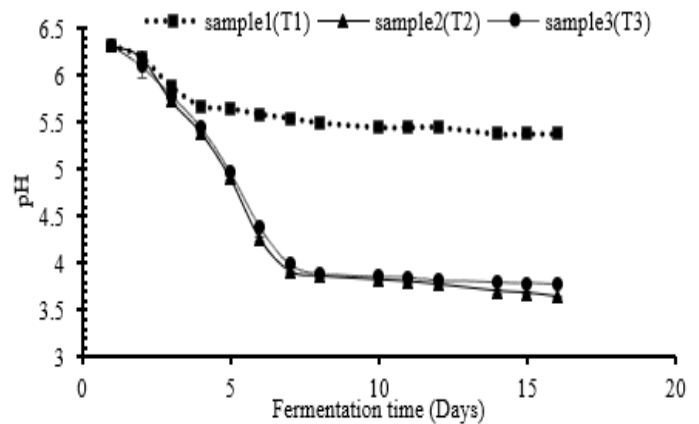


**Fig. 7.** Changes in acidity of radish pickle in three different temperatures.

Statistical analysis revealed significant differences ( $p < 0.05$ ) among most samples, except on day 1 (no significant difference among all samples) and on day 2 (no significant difference between incubation and room temperature samples). These findings align with Ghimire et al., (2020), who reported that acidity increases and pH decreases with fermentation time. Pardali et al., (2017) observed that higher fermentation temperatures (30°C) accelerate pH reduction and acidity development due to rapid growth of lactic acid bacteria. While Ji et al., (2009) reported acidity and pH of 0.99% and 4.13 at 5°C during the first ten weeks, our findings at refrigerated temperature were 0.042% and 5.38, respectively. This difference is likely attributable to our shorter fermentation time.

### 3.2.2 Influence of fermentation duration and temperature on pH

As illustrated in **Figure 8**, the pH of radish pickle stored at incubated and room temperatures steadily declined, while it remained stable under refrigeration.

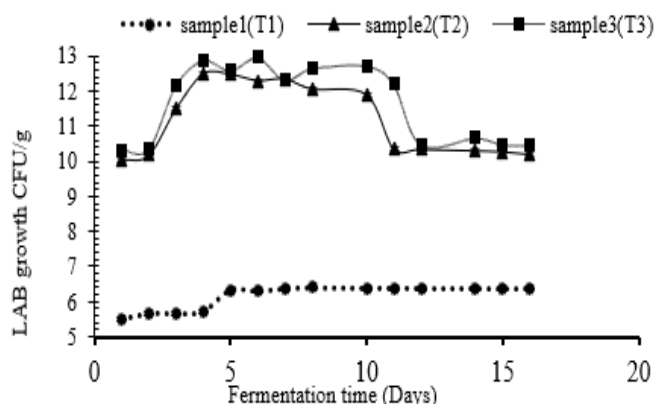


**Fig.8.** Changes in pH of radish pickle in three different temperatures

Statistical analysis revealed a significant difference ( $p < 0.05$ ) among samples overall, but no significant differences were observed on days 1 and 2. Research performed by Bautista-Gallego et al., (2020) and Aung & Eun, (2022) stated that the pH of the fermented fruits and vegetable decreases rapidly as a result of the high acid generation and low buffering capacity. Chen et al., (2023) stated that the higher pH at low temperature is due to inhibiting the growth of lactic acid bacteria and related enzyme activities. In the similar manner Joshi et al., (2014) gave conclusion that the range of percentage titratable acidity, pH and LAB count varied from 1.49–1.69 %, 2.49–3.2 and  $10 \times 10^8$  to  $6.33 \times 10^8$  during radish fermentation in  $(25 \pm 2^\circ\text{C})$ . Our result is slightly different than this which may be due to different fermentation temperature.

### 3.2.3 Effect of time and temperature on Lactic acid bacteria growth

As illustrated in **Figure 9**, lactic acid bacteria grew more rapidly at incubated and room temperatures, while only a slight, steady increase was observed under refrigeration.



**Fig. 9.** Changes in LAB growth in *radish pickle* in three different temperatures.

Statistical analysis revealed significant differences ( $p < 0.05$ ) among most samples at the 5% significance level. Karki et al., (1983) stated that the rapid growth of heterofermentative rods and homofermentative tetrads could be the cause of initial exponential increase of LAB population. In similar manner, Pardali et al., (2017) found LAB population reaching approximately 7.7 CFU/mL and reduced to 6.71 CFU/mL when fermentation was carried at 30 °C.

### CONCLUSIONS

During 16 days of fermentation, pickle acidity (% lactic acid) increased from 0.03% to 0.042% (refrigerated, T1), 1.21% (incubation, T2), and 1.178% (room temperature, T3). No significant acidity differences ( $p > 0.05$ ) were observed among samples at the end of day 1, and between T2 and T3 at the end of day 2, but significant differences ( $p < 0.05$ ) appeared from day 3 onward. The pH dropped from 6.32 log<sub>10</sub> CFU/g to 5.38 log<sub>10</sub> CFU/g (T1), 3.64 log<sub>10</sub> CFU/g (T2), and 3.78 log<sub>10</sub> CFU/g (T3), with no significant differences at day 1, but significant differences ( $p < 0.05$ ) from day 3. LAB counts rose from 4.56 log<sub>10</sub> CFU/g to 6.4 log<sub>10</sub> CFU/g (T1), 12.49 log<sub>10</sub> CFU/g (T2), and 12.96 log<sub>10</sub> CFU/g (T3), showing significant differences ( $p < 0.05$ ) across temperatures throughout fermentation. Further research is recommended to model cell growth and lactic acid production kinetics in radish pickle.

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### CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest in this work.

### DATA AVAILABILITY

The data used to support the findings of this study are available upon request from the corresponding author.

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