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Original Article

Characterization of pickled *Madan* made by *Lactobacillus plantarum* NR-MD6-18 (K2) fermentation

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Abstract

The aim of this research was to improve the quality of pickled *Madan* by *L. plantarum* NR-MD6-18 (K2) fermentation, while spontaneous fermentation was used as the control treatment. The physicochemical and microbiological quality were then monitored during fermentation at room temperature (30±2 °C) for 7 days. The main results showed that the pH in all treatments decreased after day 0 (P<0.05). The pickled *Madan* fermented by K2 provided higher levels of antioxidant activity and vitamin C than the control treatment on days 5 and 7 (P<0.05). Furthermore, the fermented acidic condition (pH 1.65-1.67) did not affect the texture parameters hardness, adhesiveness, cohesiveness, or springiness of pickled *Madan* product. By the end of fermentation the lightness had significantly decreased in both treatments, while the redness and yellowness significantly increased (P<0.05). The present investigation supports potential gastronomic applications of food science. This could be a starting point for future research aimed at developing production of an instant lactic acid bacteria (LAB) starter culture without the need for large technological investments to enhance the quality of a fermented pickled product.

Keywords: fermented pickle, *Lactobacillus plantarum* starter, antioxidant, product quality, *Garcinia schomburgkiana* pierre

1. Introduction

Fruits are nutritious sources of raw materials used in fermented food products. They are connected to human health benefits associated with nutritive molecules, non-nutritive phytochemicals, and bioactive compounds. Thus, many health policies have insisted on an increase in the consumption level of fruits, owing to their health benefits. Among the most popular fermented foods, fermented fruits are also included in several diets (Di Cagno, Coda, De Angelis, & Gobbetti, 2013; Melini, Melini, Luziatelli, Ficca, & Ruzzi, 2019). *Garcinia schomburgkiana* Pierre is an edible fruit well-known in Thailand as *Madan*. It has been widely used in medical treatments due to its antioxidant compounds. Considering its high nutritional properties and unique sour taste, *Madan* is usually processed into preserved fruit, dried fruit, or pickled fruit product (Jittanit, Ananpattana, Khunthakamon, & Khuenpet, 2018; Meechai, Phupong, Chunglok, & Meepowpan, 2018).

Fermentation is potentially of interest from a gastronomic perspective, as fermented pickled fruits are used in appetizers and served with almost every meal. Fermented pickles produced from fruits are commonly preferred by consumers because of their extended shelf life, aroma characteristics, and acceptable tastes. Pickling is an ancient gastronomic craft of preserving foods in brine and/or vinegar. This technique has been adopted by all cultures and communities over the globe, and is still typically accomplished through spontaneous fermentation. Sour fermented pickles are obtained by submerging raw materials in a dilute brine solution. Generally, lactic acid bacteria

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(LAB) grow in 7-14 days to generate significant metabolites, such as lactic acid and bacteriocin that prevent the growth of spoilage microorganisms and food pathogens (Behera, El Sheikha, Hammami, & Kumar, 2020; Chakraborty & Roy, 2018; Septembre-Malaterre, Remize, Poucheret, 2018). However, spontaneous fermentation without a starter inoculation may cause a slow long fermentation and unstable quality of the pickled products (Wouters, Grosu-Tudor, Zamfir, & De Vuyst, 2013b; Yang *et al.*, 2020).

To address both quality and safety, an inoculating starter culture is commended during fermentation of pickled products. For instance, *Lactobacillus* strains have been recommended as convenient starter cultures for fermented pickles. In prior literature, Özer and Yıldırım (2019) revealed that cabbage fermented in brine has a good potential associated with bioactive compounds and a high level of antioxidants, which is a promising beneficial aspect. Degrain, Manhivi, Remize, Garcia, & Sivakumar (2020) also demonstrated that *L. plantarum* limited the color changes and increased the antioxidant activity and total polyphenol in fermented nightshade leaves. Minh (2022) recently presented that brine fermentation pickling could improve nutritional and physicochemical profiles, and retard the growth of food pathogens and food spoilage.

In our previous collaborative work, *L. plantarum* (currently *Lactiplantibacillus plantarum*) NR-MD6-18, classified as a bacteriocin producing strain, was isolated from pickled *Madan* product in Nakhon Ratchasima province, Thailand. This strain provided a good potential starter and produced bacteriocin inhibiting food pathogens, especially *Bacillus cereus* TISTR 687 (Chanprasert, 2010; Chanprasert, & Gasaluck, 2011). When developing the quality of a pickled *Madan* product, it is significant to evaluate the potential of *L. plantarum* NR-MD6-18 for use in fermentation. *Madan* fruit have unquestionable health benefits and the application of this fruit, especially in a pickled product, should be pursued. According to academic databases, few studies have reported on *L. plantarum* activity during Thai pickled *Madan* fermentation.

Based on that background, the present study aimed to investigate the potential use of *L. plantarum* NR-MD6-18 starter to enhance the quality of a pickled *Madan* product. During fermentation for 7 days, the physicochemical quality, in terms of pH, titratable acidity, vitamin-C content, antioxidant activity, and characteristics of texture and color, were observed. The microbiological parameters including total viable count, lactic acid bacteria count, and yeast and mold counts, were also monitored.

2. Materials and Methods

2.1 Starter culture preparation

The potential starter, *L. plantarum* NR-MD6-18 (K2), was obtained from Food microbiology laboratory in Suranaree University of Technology, Nakhon Ratchasima province, Thailand (Chanprasert, 2010; Chanprasert, & Gasaluck, 2011). It was incubated in MRS broth (Himedia) at 30 ^{o}C for 48 h. The cells were then harvested by centrifugation (10,000×*g* at 4 ^{o}C for 10 min) and washed twice with 0.85% (w/v) saline, then resuspended in 0.85% (w/v) saline to approximately 9-10 log CFU/mL for further inoculums.

2.2 Pickle fermentation

Fresh *Madans* were obtained from Ban Tha Sai, Nakhon Nayok province, Thailand. They were washed with running tap water. The brine solution was prepared with cooled boiled water containing 7% salt (w/v). Pickle fermentation was then carried out in 600 ml of glass jars containing 250 g sliced *Madans* completely immersed in brine solution. Subsequently, 10% (w/v) of *L. plantarum* NR-MD6-18 was inoculated into the jar (K2), so that the final concentration of LAB in the sample was approximately 8 log CFU/g. In addition, the uninoculated cases with spontaneous fermentation served as control treatment group. These jars were sealed with water and fermented at room temperature (30±2 ^{o}C) for 7 days. Samples were collected on days 0, 3, 5, and 7 to analyze microbiological and physicochemical quality indicators.

2.3 Analysis of microbial counts

The microbial analysis was done per FDA-BAM (2001). Twenty-five grams (both of the *Madan* and fermented broth) of each sample were aseptically homogenized at medium speed for 60 s using a stomacher. Tenfold dilutions were made with 0.85% (w/v) NaCl. Subsequently, the total viable count was monitored by spreads on plate count agar (Himedia) incubated at 37 ^{o}C for 24 h. The count of lactic acid bacteria was enumerated by spreads on MRS agar (Himedia) incubated at 30 ^{o}C for 48 h. The yeasts and molds were counted by spreads on potato dextrose agar (Himedia) incubated at 30 ^{o}C for 48 h. The results are expressed in log CFU/g.

2.4 Analysis of pH and titratable acidity

The pH of pickled *Madan* was measured by using a pH meter (Fisher scientific model AB15). Prior to pH analysis, the pH meter was calibrated using standard buffer solutions for pH 4 and pH 7. Meanwhile, titratable acidity was determined as described by AOAC (2000), with the results expressed as the percentage of lactic acid.

2.5 Analysis of DPPH scavenging activity and vitamin C content

DPPH (2,2-diphenyl-1picryl hydrazyl) scavenging activity of the pickled *Madan* was determined by a spectrophotometric method (Krüzselyi *et al.*, 2020). The content of vitamin C in the pickled *Madan* was measured by an indophenol method (AOAC, 2000).

2.6 Analysis of color and texture profiles

The skin color was measured using a Chroma meter (CR-410, Konica Minolta, Japan) to record the L* (white-black), a* (red-green), and b* (yellow-blue) color coordinates of the 30 *Madans*. Meanwhile, the texture profiles of pickled *Madan* were measured by a Texture analyzer (CT3 10K, Brookfield, USA) using a 3 mm probe to puncture the mesocarp tissue at a test speed of 2.5 mm/sec. For each sample, a 7.0±0.2 mm slice was taken from the center of 30 *Madans*. The hardness, adhesiveness, cohesiveness, and springiness were also recorded.

2.7 Statistical analysis

All quantitative experiments were performed in triplicate. The results were analyzed by using one-way ANOVA in IBM SPSS statistics 23. Data are presented as mean value ± standard deviation. Duncan's Multiple Range Test was performed at a significance level of 0.05.

3. Results and Discussion

Based on the reviewed literature on pickles fermented by lactic acid bacteria, the pickling process should be performed at room temperature for 4-7 days, while the investigation of the LAB starter growth should use the end of stationary phase. In addition, the pH dropped to a final value <4.0 and the total acidity from lactic acid increase to approximately ≤1 % (Behera *et al.*, 2020; Gezginç & İnanç, 2021; Xiong *et al.*, 2016).

3.1 Microbial counts, pH, and acidity in pickled *Madan*

The pickled *Madan* fermentation was pursued with *L. plantarum* NR-MD6-18 (K2) and also with spontaneous fermentation (control). During the 7-day fermentation, the microbial growth kinetics were monitored. Figure 1A shows that the initial level of total viable count for both treatments was approximately 5.06-5.04 log CFU/g on day 0, while the maximum level of pickled *Madan* fermented with K2 was about 7.58±0.01 log CFU/g on day 3. On days 5-7, the total viable counts of pickled *Madan* fermented with K2 or with spontaneous fermentation did not differ (P>0.05). According to lactic acid bacteria counts (Figure 1B), the initial count for pickled *Madan* inoculated with LAB starter was approximately 8.35±0.07 log CFU/g while at the end of fermentation (day 7) it was higher than that with spontaneous fermentation (P<0.05). However, the growth of lactic acid bacteria tended to decrease after 5 days of fermentation. This might be related to the investigation of Wang *et al.* (2022), who revealed that the LAB starter cultures of *Leuconostoc mesenteroides*, *Levilactobacillus brevis*, and *Weissella cibaria* rapidly decreased after 3 days in the process of paocai fermentation, thereby resulting in the end of stationary phase. In Figure 1C, the growth of yeast and mold in each treatment was competitive in days 0 and 3, but a decrease in yeast and mold counts was observed on day 7. This phenomenon could be interpreted so that the microbiota and indigenous LAB could grow kinetically under stress environment throughout the fermentation in 2-5-7.0 % brine solution (Susilowati, Laia, & Purnomo, 2018). Such *Madan* substrate and parameters of brine fermentation relate to the growth factor of LAB and their stress response. Yapwattanaphun, Subhadrabandhu, Sugiura, Yonemori, and Utsunomiya (2000) reported that *Madan* contains both protein (0.3 g/100g) and carbohydrates (6.5 g/100g). It was assumed that these nutrient-like intrinsic factors could enhance the primary and secondary metabolism by LAB (Bautista-Gallego *et al.*, 2020). The changes in percentage of lactic acid and pH are shown in Figures 2A and B, respectively. The highest percentage of lactic acid in pickled *Madan* fermented with K2 was found on days 3-7 of fermentation, but not so for the control treatment. Meanwhile, the pH of both treatments decreased after 0 day of fermentation, which had about 1.65-1.67. This was associated with the fact that LAB converted the fermentable raw materials, such as monosaccharides and/or disaccharides, into organic acid, especially lactic acid, which contributes to controlling the specific quality of fermented foods in brine solution (Canon, Nidelet, Guédon, Thierry, & Gagnaire, 2020; Kanpiengjai, Nuntikaew, Wongsanittayarak, Leangnim, & Khanongnuch, 2022; Penland *et al.*, 2022).

3.2 DPPH scavenging activity and vitamin C in pickled *Madan*

According to the scientific theory of metabolites produced from lactic acid bacterial fermentation, antioxidant compounds include but are not limited to flavonoids, anthocyanins, and phenolic compounds. Specific compound categories such as exopolysaccharides, peptides, amino acids, fiber, and ascorbic acid also contribute to the DPPH free

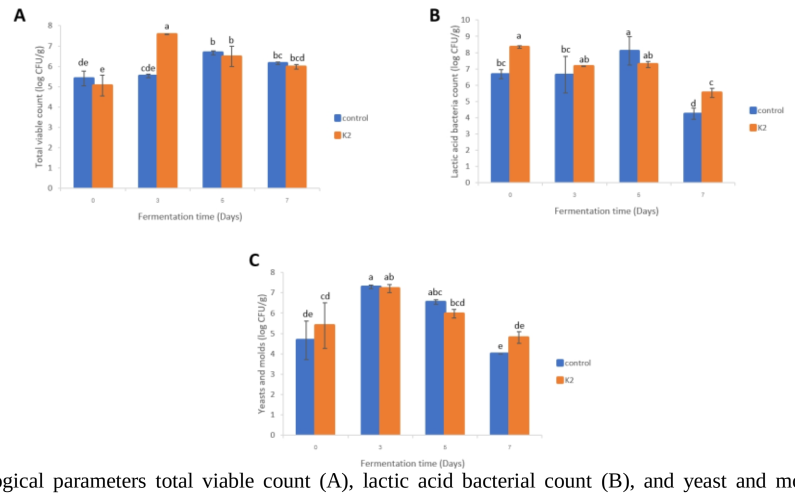

Figure 1. The microbiological parameters total viable count (A), lactic acid bacterial count (B), and yeast and mold counts (C) of pickled *Madan* during fermentation, Results are presented as mean ± standard deviation. Means in each parameter followed by different lowercase letters are significantly different (P<0.05) according to DMRT.

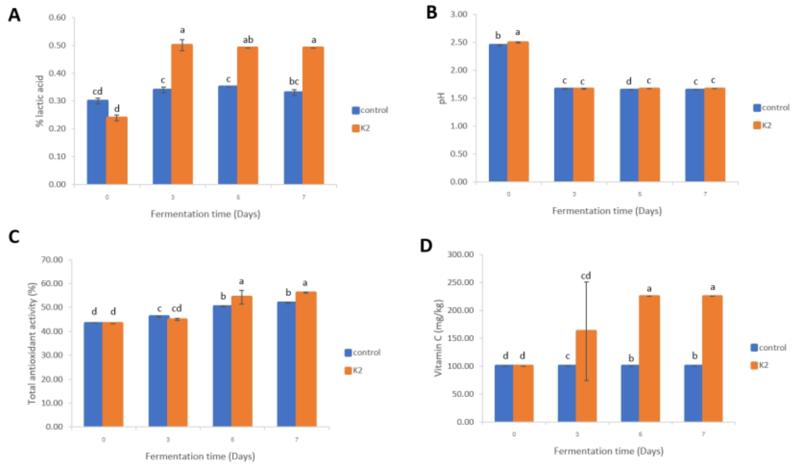

Figure 2. The percentage of lactic acid (A), pH (B), total antioxidant activity (C), and vitamin C content (D) of pickled *Madan* during fermentation, Results are presented as mean ± standard deviation. Means in each parameter followed by different lowercase letters are significantly different (P<0.05) according to DMRT.

radical scavenging activity. In addition, these metabolites could be classified as phytochemicals characteristic of fermented fruits and vegetables (Adesulu-Dahunsi, Jeyaram, Sanni, & Banwo, 2018; Chuah, Tang, Ang, & Tan, 2021; Hunaefi *et al.*, 2013; Zhou *et al.*, 2021). Regarding the phytochemical properties of pickled *Madan* fermented with K2, the antioxidant activity and vitamin C increased on days 5 and 7 of fermentation (Figures 2C-D) and were higher than the levels with spontaneous fermentation (P<0.05). This indicates that the LAB starter fermentation is associated with an increase in the content of phytochemical substances, especially of vitamins and antioxidant activity. The starter culture inoculation is interesting, in this context, for promoting the nutritional value of fermented food products. The effects of LAB on antioxidant activity could be explained by the liberation of simple phenolic compounds after enzymatic hydrolysis of polymerized phenolic compounds during acid fermentation. Meanwhile, many LAB have an antioxidant activity and possess enzymatic and non-enzymatic antioxidative mechanisms to minimize the generation of reactive oxygen species (Hur, Lee, Kim, Choi, & Kim, 2014). Likewise, Wouters *et al.* (2013a) found that Leek leaves (*Allium ampeloprasum* var. *porrum*) fermented with *Lactobacillus sakei* IMDO 1358 had a positive influence on antioxidant capacity when compared to spontaneously fermented leek. Degrain *et al.* (2020) also revealed that African nightshade (*Solanum retroflexum* Dun) fermented with *L. plantarum* 75 significantly decreased the pH, and increased the concentration of ascorbic acid after 3 days.

3.3 Color characteristics of pickled *Madan*

Product appearance and in particular its color during the fermentation (Figures 3-4) are the most significant quality characteristics that affect consumer perception of a food product. The results revealed that the pickling conditions affected color. At the end of fermentation, the lightness (L*) in both treatments had significantly decreased but the redness (a*) and yellowness (b*) had significantly increased (P<0.05), indicating that the color of pickled *Madan* became more green-yellow and darker than that of fresh *Madan*. This

Figure 3. Appearance of each treatment of pickled *Madan* during fermentation

phenomenon is in agreement with the lactic acid produced by LAB during fermentation, which indicates the degradation of chlorophylls into pheophytins and pheophorbides (Koca, Karadeniz, & Burdurlu, 2007; Shaaban & Abd El Mageed, 2020; Shang *et al.*, 2022). Sangija, Martin, and Matemu (2022) revealed that LAB fermentation could reduce the content of chlorophyll in pickles. The acidic metabolites include lactic and acetic degraded chlorophyll a and b. After that, pH changes produced new compounds such as brown pigments (*O*-quinones), a carotenoid with 5,8-epoxide groups, Mg-free chlorophyll derivatives, or phenolic compounds' chemical oxidation, resulting in a decrease in the chlorophyll content (Degrain *et al.*, 2020; Ramírez, Gandul-Rojas, Romero, Brenes, & Gallardo-Guerrero, 2015). The a* and b* in all treatments on day 3 had significantly decreased (P<0.05) when compared to day 0. This result was related to the research of Ramírez *et al.* (2015), who reported that the a* and b* of non-pasteurized olives decreased after being placed in sterile acidified brine solution.

3.4 Texture profiles in pickled *Madan*

The texture profiles of pickled product have an influence on consumer acceptance. A change in the texture of pickled product might be due to the loss of cell wall strength and adhesion, due to the change in pH during pickle fermentation (Shang *et al.*, 2022). The hardness, adhesiveness, cohesiveness, and springiness of all pickled *Madan* during fermentation are displayed in Figure 5. The

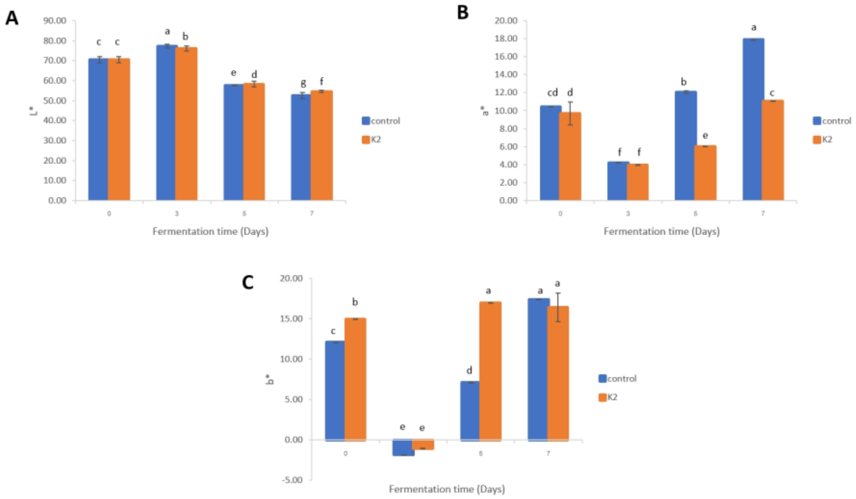

Figure 4. The color coordinates L* (A), a* (B), and b* (C) of pickled *Madan* during fermentation, Results are presented as mean ± standard deviation. Means in each parameter followed by different lowercase letters are significantly different (P<0.05) according to DMRT.

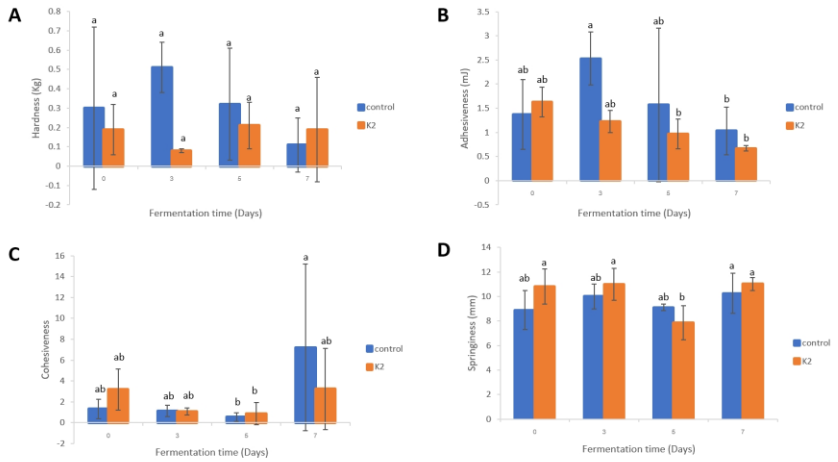

Figure 5. The texture parameters hardness (A), adhesiveness (B), cohesiveness (C), and springiness (D) of pickled *Madan* during fermentation, Results are presented as mean ± standard deviation. Means in each parameter followed by different lowercase letters are significantly different (P<0.05) according to DMRT.

texture parameters of the treatments on day 7 did not differ from those on day 0 or between the treatments (P>0.05). Thus, the acidic pickling conditions did not affect the texture characteristics of pickled *Madan*, although previous research revealed that pickling process could lead to a degradation of fruit and vegetable texture, due to the solubilization and depolymerization of the cell wall pectin (Zheng *et al.*, 2013). As a result, we assume that the microbial pectinolytic enzyme activity was not capable of degrading the cell wall of *Madan* substrate.

4. Conclusions

The present work is important in developing the quality of pickled *Madan* products by testing the potential of *L. plantarum* NR-MD6-18 (K2) inoculated fermentation. The results on days 5-7 revealed that pickled *Madan* fermented with K2 provided better levels of phytochemical properties, particularly antioxidant activity and vitamin C. In addition, the fermented acidic conditions did not affect the texture parameters of pickled *Madan* product. Given these results, the developed product could be easily self-produced on household or restaurant levels. It is further possible to achieve producing fermented pickled fruit by instant LAB starter culture, taking into account the trends in the field of gastronomy in relation to food sciences that do not depend on large technological investments to improve the quality of fermented pickles.

Further studies should be devoted to validating the association between sensory evaluation and flavor compounds along with changes in microbial community during fermentation.

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