

The Effect of a Mixed-Starter Culture of Lactic Acid Bacteria on the Characteristics of Pickled Orange-Fleshed Sweet Potato (*Ipomoea batatas* L.)

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In this study, fermentation process was carried out on orange-fleshed sweet potato cubes to produce sweet potato pickle using a mixed culture of *Lactobacillus plantarum* and *Leuconostoc mesenteroides* at 30 °C over 12 d period. Spontaneous fermentation was also performed as a control. Samples were withdrawn at various time intervals for analyses of reducing sugar content, total number of lactic acid and non-lactic acid bacteria, lactic acid concentration, pH, and sensory attributes. The results showed that using a mixed culture of *L. plantarum* and *L. mesenteroides* could greatly reduce contamination of non-lactic acid bacteria, retaining low amount of reducing sugar, rapidly producing lactic acid and consequently decreasing pH value of the pickle, as well as giving better sensory score. After 12 d of fermentation, sample of pickle inoculated with mixed culture showed the following characters: total lactic acid content 0.5%, total lactic acid bacteria 8.46 log₁₀ CFU mL⁻¹, total non-lactic acid bacteria 1 log₁₀ CFU mL⁻¹, total reducing sugar 0.84 g L⁻¹, texture 64.92 mm 50 g⁻¹ s⁻¹, and hedonic sensory score for both taste and aroma 4 (like) in a scale of 5. These results indicated the potential ability of the mixed culture of lactic acid bacteria to improve the quality of the pickle fermented spontaneously.

Key words: fermentation, lactic acid bacteria, mixed-starter cultures, pickle, sweet potato

Pada penelitian ini, potongan ubi jalar kuning difermentasi untuk memproduksi pikel menggunakan kultur campuran *Lactobacillus plantarum* dan *Leuconostoc mesenteroides* pada 30 °C selama 12 hari. Fermentasi spontan juga dilakukan sebagai kontrol. Parameter yang diamati meliputi gula reduksi akhir, total bakteri asam laktat (BAL), total non-BAL, konsentrasi asam laktat, pH, tekstur, dan sensori. Hasil penelitian menunjukkan bahwa penggunaan kultur campuran *L. plantarum* dan *L. mesenteroides* dapat menekan pertumbuhan kontaminan non-BAL, menyisakan sejumlah kecil gula reduksi, menghasilkan asam laktat dengan cepat sehingga menurunkan pH secara cepat, serta menghasilkan sensori yang lebih baik. Karakteristik pikel setelah 12 hari fermentasi adalah sebagai berikut: total asam laktat 0,5%, total BAL 8,46 log₁₀ CFU mL⁻¹, total non-BAL 1 log₁₀ CFU mL⁻¹, total gula pereduksi sisa 0,84 g L⁻¹, tekstur 64,92 mm 50 g⁻¹ s⁻¹, dan nilai sensori untuk rasa dan aroma masing masing adalah 4 (suka) dari 5 skala, untuk pikel dengan perlakuan kultur campuran. Hasil ini menunjukkan bahwa penggunaan kultur campuran BAL pada fermentasi pikel ubi jalar dapat memperbaiki kualitas pikel yang difermentasi secara spontan.

Kata kunci: bakteri asam laktat, fermentasi, kultur campuran pikel, ubi jalar

Orange fleshed sweet potatoes have important nutritional values because of high concentration of β-carotene that functions as provitamin A. The carotenoid content of orange-fleshed sweet potatoes has been reported around 18 mg/100 g fresh weight (Panda *et al.* 2007). This potential character of orange-fleshed sweet potatoes should be responded by many food manufacturers to produce a healthy food. An alternative to diversify the sweet potato utilization is to use it as a raw material for pickle processing.

Most pickled vegetables are processed through spontaneous fermentation without inoculum addition. For this reason, the result of the process is often very unpredictable due to the diversities of the initial microflora. This has caused inconsistency in the

quality of the pickle, off-flavoring, and the growth of different types of microorganisms. Fresh vegetables may contain various wild microflora, dominated by non lactic acid bacteria responsible for spoilage (Fleming 1982). An alternative to control the fermentation process is by adding lactic acid bacteria (LAB) cultures during pickle processing. LAB could rapidly produce lactate and other organic acids, and accumulate them in the raw materials. Furthermore, they can also produce various metabolites that contribute to the development of some desirable characteristics such as visual appearance, texture, shelf-life, acceptability, and safety (Holzapfel 1997; Holzapfel 2002; Leroy and Vuyst 2004; Dalie *et al.* 2010). Some researchers have shown potential benefits of using starter cultures in fermentation, for example in the productions of low-salt sauerkraut, sauerkraut,

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sauerkraut juice, and sausages (Tolonen *et al.* 2002; Wiander and Ryhanen 2005; Johanningsmeier *et al.* 2007; Rai *et al.* 2006; Hu *et al.* 2007).

Research on the production of pickled sweet potatoes has not been done as intensively as on pickled cucumber. Several groups have reported the fermentation of pickled sweet potatoes without inoculation with a mixed culture (Panda *et al.* 2007; Panda *et al.* 2009; Sivakumar *et al.* 2010; Yuliana and Nurdjanah 2009; and Yuliana *et al.* 2010). So far there is no report mentioning the effect of inoculation with bacterial cultures commonly used for pickle processing, such as *Leuconostoc mesenteroides* and *Lactobacillus plantarum*, on the characteristics of pickled sweet potatoes. In this study, we compared the effects of adding *L. mesenteroides* and *L. plantarum* cultures on pickled sweet potatoes to pickle that had been processed spontaneously (without culture addition), to determine whether the addition of these starter cultures might have given positive effects on the fermentation.

MATERIALS AND METHODS

Preparation of Starter Culture. Pure cultures of *L. plantarum* FNCC 0123 and *L. mesenteroides* FNCC 0023 were purchased from Food and Nutrition Inter University Center (PAU) Universitas Gadjah Mada. The starter was prepared by inoculating one loop of pure cultures of *L. mesenteroides* FNCC 0023 and *L. plantarum* FNCC 0123 into tubes containing 10 mL of sterile MRS Broth and incubated at 37 °C for 24 h. One mL of these cultures was then sub cultured twice (using pour plate and streak plate methods) in MRS agar at 37 °C for 24 h. Single colony was picked up and inoculated twice into a centrifuge tube containing 10 mL MRS broth and incubated for 24 h 37 °C for the first and 48 h 37 °C for the second incubation. Finally, the broth of each culture of *L. plantarum* and *L. mesenteroides* were combined and harvested by centrifugation (IEC Centra CL2 Centrifuge) at 349 x g for 5 min. The amount of cells in this preparation was approximately 2×10^6 CFU mL⁻¹.

Brine Solution Preparation. Brine solution was prepared by mixing 15 g NaCl with 2.5 g sucrose, and then dissolved in 250 mL distilled water. The brine solution were heated using microwave oven (Sharp) at high level setting for 6 min to achieve 60 °C, and then left for 7 min in a container containing warm water (35-40 °C). This solution was distributed into 150 mL of fermenting containers.

Sweet Potato Pickle Processing. Orange-fleshed sweet potato samples were obtained from Pasar Gintung, a local market at Bandar Lampung, Province of Lampung-Indonesia. The sweet potatoes were washed, peeled, and cut into cubes (1x1x1 cm³). The potato cubes (40 g) were put into a 150 mL-fermenting container, then it was made up to 150 mL volume with the brine solution. The fermenting containers containing the samples were pasteurized using a microwave oven (Sharp) at high level setting for 10 min to reach temperature around 72-73 °C, and then they were left at room temperature to cool. Each sample was inoculated with the mixed culture of *L. plantarum* and *L. mesenteroides* at level of 2×10^6 CFU mL⁻¹. In addition, fermenting container without inoculum addition was also prepared as a control. The fermentation was conducted at room temperature (30 °C) for 12 d. The experiment was designed in four replicates.

Evaluation of the Characteristics of the Pickled Sweet Potatoes. Evaluation on the physicochemical properties was performed on lactic acid by titration method (AOAC 1980), reducing sugar using Nelson-Somogyi method (Somogyi 1952), starch, and texture. The starch content was determined enzymatically at the beginning and the final day of fermentation. The sample was homogenized, gelatinized, and subjected to α -amylase and amyloglucosidase (Sigma-Aldrich) digestion for 20 min at 55 °C then the enzyme activity was stopped by adding few drops of tri chloroacetic acid (TCA) and centrifuged at 1398 x g (slightly modified from Nurdjanah 2008). The glucose in the supernatant was quantified using phenol-sulfuric acid method (Dubois *et al.* 1956). Total starch content was calculated by multiplying the result by 0.90 (AOAC 1980). Texture was determined according to Nurdjanah *et al.* (2007) using penetrometer (Precision, Scientific Petroleum Instruments) and reported as the depth of the sweet potato tissue penetrated by the cone probe when applied with 50 g loading force per second (mm 50 g⁻¹ s⁻¹).

Microbial growth during fermentation was evaluated by counting the total lactic acid bacteria (LAB) and non-lactic acid bacteria (non-LAB) using total plate count method. Appropriate dilutions were placed on duplicate plates of MRS agar medium (Oxoid) containing 0.1% (w/v) CaCO₃. The cultures were then incubated at 30 °C for 48 h. LAB was identified by the presence of clear zones around the colonies; otherwise they were identified as non-lactic acid bacteria.

The sensory evaluation was conducted at day 12 of

fermentation according to method by Meilgaard *et al.* (2007) and performed by a sensory panel composed of 18 panelists. A five-point hedonic scale, varying from dislike very much (score 1) to like very much (score 5) was used. Each of 18 panelists evaluated all samples in randomized order of presentation and evaluated the pickle in relation to taste and aroma using the score sheet for hedonic testing.

RESULTS

The increase of cell numbers (represented in CFU mL⁻¹) produced during 12 d fermentation with prior inoculation of *L. plantarum* and *L. mesenteroides mixed culture* is shown in Fig 1A-1B. During 12 d of fermentation, the populations of these strains increased approximately 5 log cycles, while the number of LAB in control increased only approximately 2 log cycles. The total number of LAB in the spontaneous fermentation was lower than those in batch with mixed

inoculum throughout the 12 d of fermentation.

The lactic acid was already observed at early stage of fermentation and rapidly increased to an amount of 0.3% (v/v) on day three of fermentation in batch with mixed inoculum. However, inspontaneous fermentation, rapid increase was observed on day six of fermentation (Fig 2A). Lactic acid content increased linearly with fermentation time from 0.03 to 0.27% and 0.007 to 0.52% at rate 0.06 and 0.11% for 12 d in spontaneous and mixed inoculum batch, respectively. The final lactic acid content of the pickle inoculated with mixed culture and spontaneous fermentation was 0.52 and 0.27%, respectively.

The reducing sugar increased quadratically with fermentation time from 0.46 to 0.71 and 0.44 to 0.84 g L⁻¹ in both spontaneous and mixed inoculum pickle, respectively, during 12 d fermentation (Fig 2B).

In contrast to the patterns observed on reducing sugar and starch utilization, during 12 d of fermentation the texture of the pickles seemed to alter

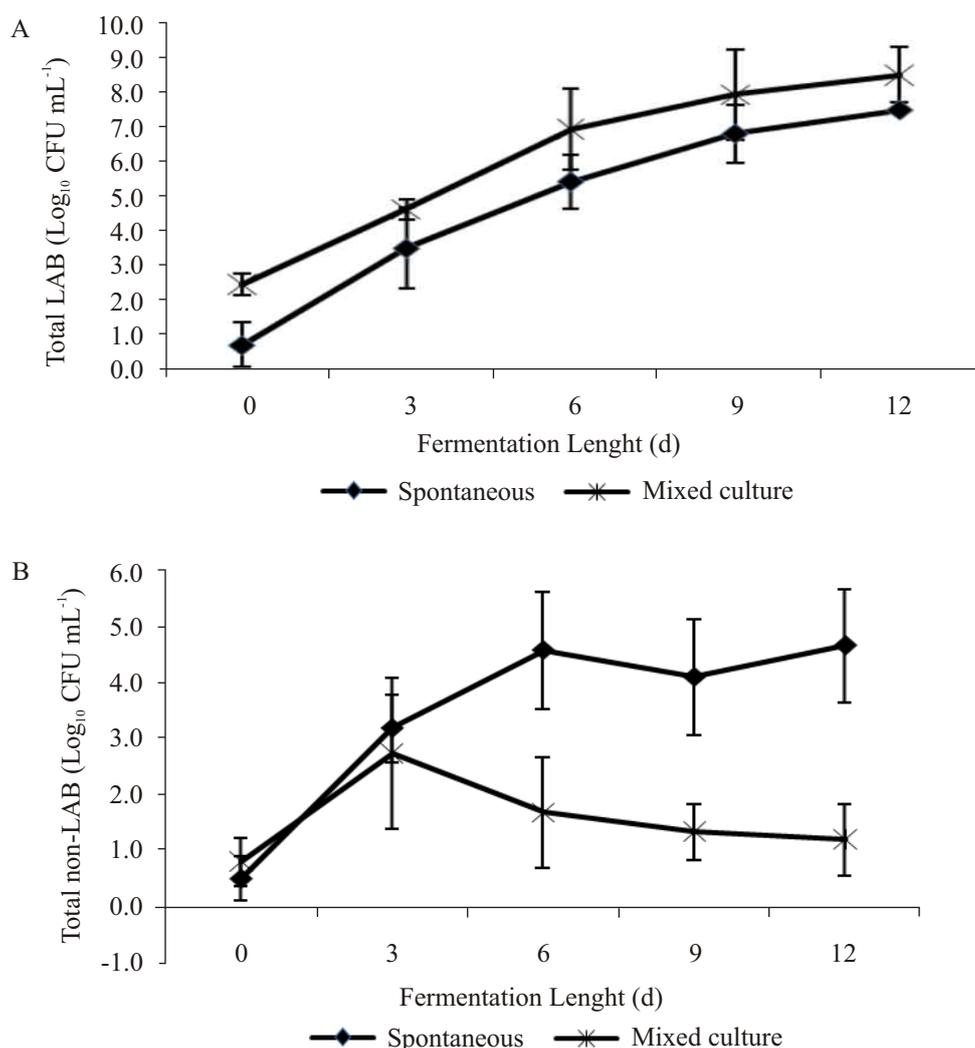


Fig 1 Total LAB (A) and non-LAB (B) population during 12 d fermentation.

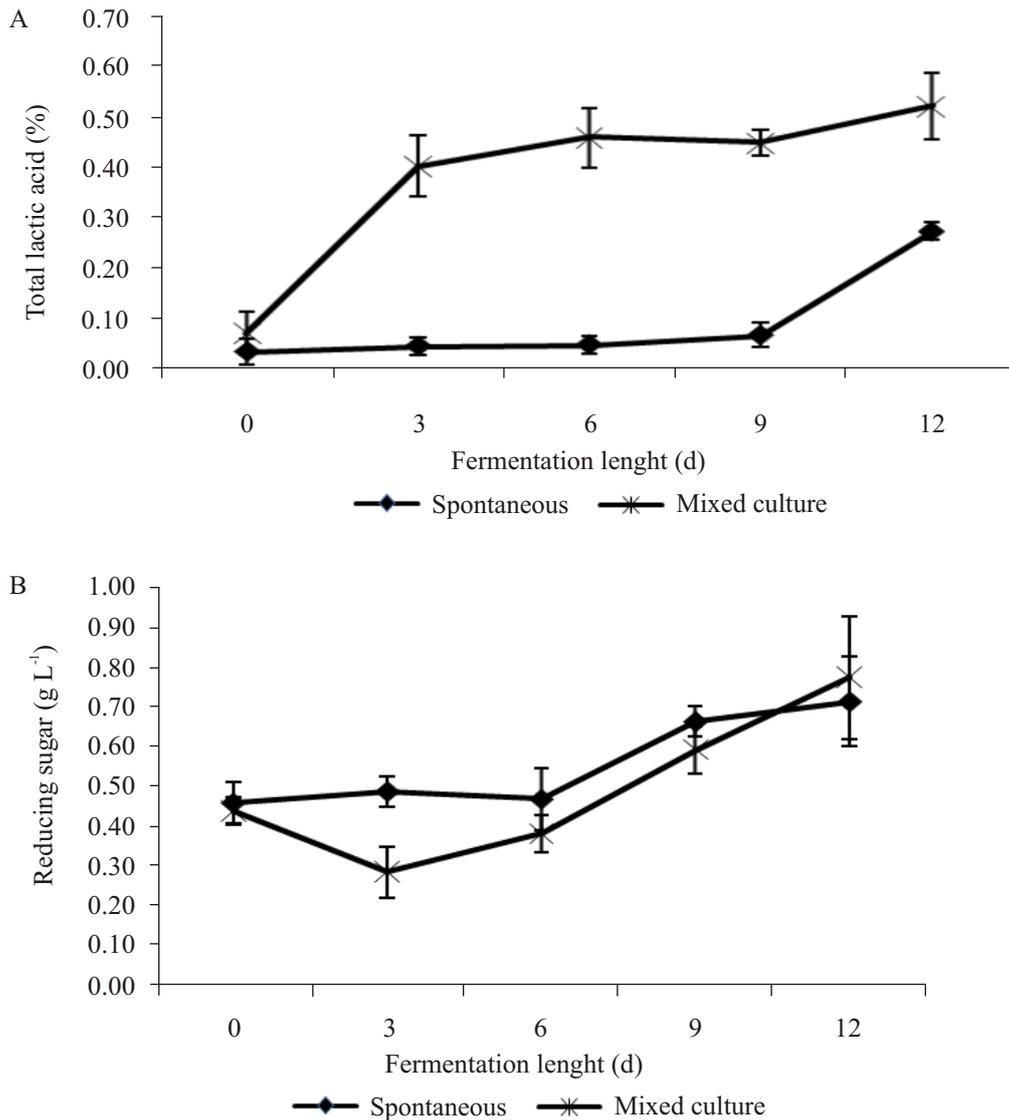


Fig 2 Total lactic acid (A) and reducing sugar (B) production during 12 d fermentation.

in similar pattern, whether inoculated with mixed culture or not (Fig 3). The firmness of the pickled vegetable decreased quadratically with fermentation time, as indicated by the increasing depth the probe could penetrate the tissue, that is from 43.16 to 59.31 in spontaneous fermentation and 43.71 to 64.92 mm 50 g⁻¹ s⁻¹ in pickled that had been inoculated with mixed culture. The final texture of pickle with mixed culture

and spontaneous fermentation ranged 50-60 mm 50 g⁻¹ s⁻¹, indicating that the texture of the pickle cube was slightly softer.

The sensory evaluation showed that panelists preferred the pickle that had been inoculated with mixed culture. The scores for taste and aroma of the pickle inoculated with mixed culture were 3.9 and 4.2 (liked), while scores of those with spontaneous fermentation

Table 1 The starch content of fresh and pickled sweet potato

Treatments	Starch content (%)		
	Before fermentation	After fermentation	Efficiency (%)
Fresh sweet potato*	29.11±1.11	-	-
Spontaneous	29.33±1.07	25.43±0.09	13.30
Inocultaed with mixed culture	26.84±0.31	21.06±1.91	21.57

*moisture content of fresh sweet potato was 81%

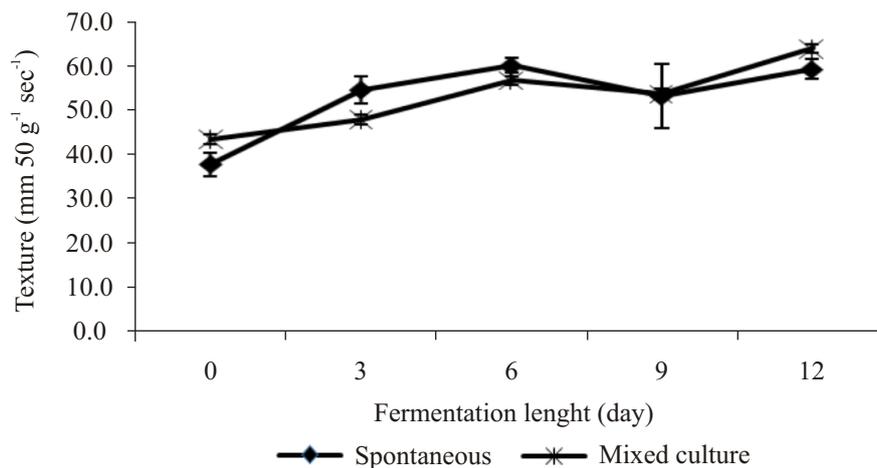


Fig 3 Change of texture during 12 d fermentation.

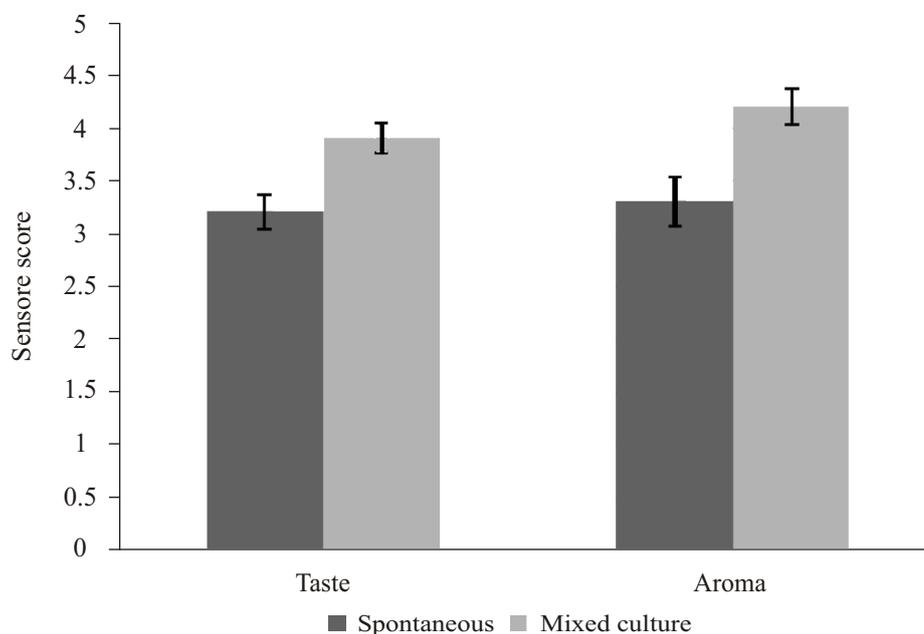


Fig 4 Hedonic sensory score at day 12 fermentation of pickle with spontaneous fermentation and mixed culture inoculation. Score 1=dislike very much, 2=dislike, 3=moderate (neither like nor dislike), 4=like, 5=like very much.

were 3.2 and 3.3 (neither liked nor disliked).

DISCUSSION

The addition of LAB culture significantly affected the total LAB, total non-LAB, lactic acid content, and reducing sugar during fermentation, as well as the sensory of final product. Throughout the 12 d of fermentation the total population of LAB in the spontaneous fermentation was lower than those in batch inoculated with mixed culture. The higher population of LAB in the pickle that had been inoculated with mixed culture greatly reduced the total contaminating non-LAB. Consequently, the population of total non-LAB in the batch with spontaneous

fermentation was higher than those in the batch that was inoculated with mixed cultures (Fig 1 B). The high population of LAB at the initial stage of fermentation had an important role in inhibiting growth of contaminants even after fermentation had started (Hu *et al.* 2007; Rai *et al.* 2006). In order to control the fermentation, LAB starter cultures have been used in the fermentations of sauerkraut, olives, cucumber, sausages, and other fermented products (Fleming *et al.* 2001; Hu *et al.* 2007; Rai *et al.* 2006; Zdolec *et al.* 2008). The different LAB population densities could affect the amount of lactic acid produced. It has been found that the faster the production of lactic acid, the faster the pH decreased (Tolonen *et al.* 2002; Wiander and Ryhanen 2005), thus the faster the completion of

fermentation.

Reducing sugar remained relatively lower in the culture inoculated batch than that in spontaneous batch throughout the fermentation. This was probably due to a faster growth rate of starter culture. LAB produced amylase enzyme which rapidly degraded starch to simple sugars that could then be utilized for lactic acid production (Petrova *et al.* 2013). Therefore, rapid decrease of reducing sugar in substrate (Fig 2B) was observed in the LAB culture inoculated batch. The degradation of starch was indicated by the decrease of starch content in the pickled sweet potatoes after fermentation (Table 1). Amylase enzyme was reported to present naturally in sweet potatoes (Takahata *et al.* 1995; Hagenimana *et al.* 1994; Krishnan *et al.* 2010) and produced by LAB as well (Reddy *et al.* 2008; Petrova *et al.* 2013). This enzyme was able to rapidly degrade starch to a simple sugar such as glucose.

After day 12, the texture of the culture inoculated and spontaneously fermented pickled potatoes were both slightly soft. It has been documented that the texture is influenced by the main components of plant cell wall polysaccharides, such as cellulose, pectin, and matrix polysaccharides that are composed of interconnected hemicelluloses bound to cellulose microfibrils (Imam *et al.* 2005). This structural components give the cell wall its rigidity. It is assumed that enzymes produced by LAB and non-LAB during fermentation along with the enzymes present in the sweet potatoes were to be the major contributors to this cell wall degradation and caused the disruption of the texture of the sweet potatoes pickle.

Lactic acid bacteria and other microbes are able to produce depolymerizing enzymes that act on cell-wall components (Marin-Rodriguez *et al.* 2000; Mokemiabeka *et al.* 2011) and on starch (Petrova *et al.* 2013), thus destroying the integrity of the plant tissues. Enzymatic tissue softening caused by microbial agents has been reported in pickled cucumber (Maruvada and McFeeters 2009) and other fruits and vegetables containing pectin (Duvetter *et al.* 2009). However, the depolymerizing enzymes contributing to the softening of the pickle might have come from the sweet potatoes. It has been found that pectin esterase, pectin methyl esterase (PME), α and β amylase were natural enzymes present in sweet potatoes (Sunmola and Bukoye 2011; Binner *et al.* 2000; Hagenimana *et al.* 1994; Ikemiya and Deobald 1966). However, the activity of PME could be inhibited by rapid pH reduction (Turk 1989). This might be the rational reason why the slight softening was observed in both batch fermentations.

The addition of LAB caused a rapid acid accumulation in the raw material, with the production of lactic and several organic acids as well as other various metabolites. Furthermore, the presence of LAB culture could suppress the total non-LAB contaminants contributing to off-flavor of the pickle. Consequently, inoculating the pickle with mixed LAB culture helped to develop good sensory properties on the aroma and the tastes. The benefits of LAB culture addition have been reported by some authors. The mixed cultures containing *L. mesenteroides* and *L. plantarum* gave sauerkraut and sauerkraut juice the most desirable sensory characteristics (Wiander and Ryhanen 2005). No significant off-flavor was observed on salted cabbage inoculated with *L. mesenteroides* at the end of the fermentation, sauerkraut which had been inoculated with this strain was more uniformly fermented in comparison to the one produced by spontaneous fermentations (Johanningsmeier *et al.* 2007).

In conclusion, the results indicated the positive effect of a mixed culture of *L. plantarum* and *L. mesenteroides* on the safety and sensory quality (aroma, taste, and texture) of pickled orange-fleshed sweet potato. The addition of these LAB cultures significantly suppressed the total of non-LAB as contaminant that could contribute to the off-flavor of the pickle. This finding supported the previous similar studies on fruit and vegetable fermentation.

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