

## Effects of Lactic Acid Bacteria Inoculated Fermentation on Pickled Cucumbers

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**Abstract:** The aim of this study was to determine the effects of Lactic Acid Bacteria (LAB) fermentation on the texture and organic acid of pickled cucumbers. Texture and sensory evaluation as well as a microscopic observation were performed to study the textural differences among fresh cucumber, Spontaneous fermentation (SF) cucumber and LAB Inoculating Fermentation (LABIF) cucumber. Accumulation of seven organic acids i.e., oxalic, tartaric, malic, lactic, acetic, citric and succinic acid during cucumber pickling were also studied. The disruption extent of the middle lamella in SF cucumber displayed more obviously than that in LABIF cucumber, implying that LABIF contributed to keep the cucumber original structure intact. Based on the organic acid accumulation pattern, in SF LAB and Acetic Acid Bacteria (AAB) fermented simultaneously, while in LABIF LAB fermented beforehand thus being in dominant position, then AAB fermented vigorously in an acidic condition created by LAB. The acetic acid accumulatoin pattern could be regarded as the distinctive feature between SF and LABIF. The organic acids produced in LABIF were higher than that in SF. The final score of sensory evaluation combining texture analysis demonstrated that LABIF overmatched SF. It was concluded that LABIF could obviously enhance the quality of pickled cucumber and overwhelming SF, due to LABIF more beneficial to keep the cucumber original structure intact and organic acids accumulation.

**Keywords:** Cucumber, Lactic Acid Bacteria (LAB), organic acid, pickling, texture

### INTRODUCTION

Cucumber (*Cucumis sativus*) is an important vegetable in China. With the prevalent trend of Kimchi, Paocai and Sauerkraut, pickled cucumber has been attracting more and more attention and becoming one of the most famous pickling vegetables.

Texture of pickled vegetables was one of the most important factors determining consumer's eating satisfaction (Kohyama *et al.*, 2009; Buscher *et al.*, 2011; Suojala-Ahlfors, 2005). To evaluate the texture of cucumbers objectively, puncture test was used by many researchers to quantify the firmness (Sakurai *et al.*, 2005; Sakata *et al.*, 2011). However, it is not easy to characterize the texture by a simple puncture test for the reason that cucumber is highly heterogeneous and anisotropic in mechanical properties within a fruit (Kohyama *et al.*, 2009). Therefore, mechanical tests together with direct human measurements could illustrate cucumber texture comprehensively. Using a texture analyzer can objectively evaluate many samples without any reduction in accuracy and sensory

evaluation with trained panel members has the advantage of describing intuitively the texture characteristics during chewing. Besides, microscopic observation of pickled cucumber tissue could show intuitively the characteristic of texture.

With the increasing requirement for pickled vegetables with high-quality and good flavor, the studies on flavor components during pickling have been paid more attention. Organic acids strongly influence the organoleptic properties of vegetables, particularly when considering flavour, color and aroma (Flores *et al.*, 2012). Therefore, determination of organic acids provides a way to check and control the fermentation processes, as they contribute to flavor balance, chemical stability and microbial control (Avenozza *et al.*, 2006; Pereira *et al.*, 2010).

To evaluate organic acids, several methods have been reported, such as enzymatic methods (Mataix and de Castro, 2001), spectrophotometry methods (Rebelein, 1961), thin layer chromatography (TLC) (Ryan and Dupont, 1973), gas chromatography (Jham *et al.*, 2007; Yang and Choong, 2001), ion

chromatography (Masson, 2000) and capillary electrophoresis methods (Mato *et al.*, 2006, 2007). Besides, HPLC has become more and more popular for separation and quantification of organic acids because of its simplicity, speediness and stability (Fontannaz *et al.*, 2006; Hernandez *et al.*, 2006).

A great part of reported analyses using HPLC to determine main organic acids were in allusion to wine (Castellari *et al.*, 2000; Kerem *et al.*, 2004), fruit juice (Qureshi *et al.*, 2011; Raghav *et al.*, 2012) or raw material of other fruits and vegetables (Rodriguez *et al.*, 2008; Eyéghé-Bickong *et al.*, 2012; Erro *et al.*, 2009; Flores *et al.*, 2012). Using HPLC to evaluate organic acids in fermented vegetables, especially cucumbers remain great attractive.

Spontaneous Fermentation (SF) is still the main method for the preparation of Kimchi, Paocai and Sauerkrant products both in homemade and industrial processes (Xiong *et al.*, 2012). However, it may lead to unsafety of the products due to its complex microorganisms, mainly spoilage of fermented vegetables associated with an increase in brine pH, Nitrite Content (NC) and unpleasant odors (Franco *et al.*, 2012; Yan *et al.*, 2008).

Lactic Acid Bacteria (LAB) produce metabolites such as lactic acid to act as bio-preservatives by inhibiting spoilage microorganisms and modify the intrinsic properties of food and bacteriocin (e.g. nisin) and hydrogen peroxide which can inhibit other microorganisms effectively (Garcha and Natt, 2012; Aslam *et al.*, 2011). LAB are generally regarded safe and can enhance shelf life when used in food fermentation (Cizeikiene, 2013). Pickled products by LAB Inoculated Fermentation (LABIF) have unique flavor and great healthful effects (Choi *et al.*, 2013).

Cucumber is an important raw vegetable material for Paocai production in China. Distribution and succession of LABs in cucumber have attracted special attention (Chen *et al.*, 2012; Singh and Ramesh, 2008). Others studied microorganisms that related to fermentation process aiming to obtain end products with safety and high quality (Maegan and Ilenys, 2009; Breidt and Caldwell, 2011). Besides, researches attempted to add calcium chloride in cucumber fermentation brines to keep crispness without using alum or made efforts to adapt commercial tank yard fermentations without sodium chloride (McFeeters and Perez-Diaz, 2010; Maruvada and McFeeters, 2009; Buscher *et al.*, 2011). Improving pickling cucumbers quality remains great attractive. The objective of this study was to evaluate and compare the effects on pickling cucumbers quality of SF and LABIF, from the aspects of firmness, sensation and organic acids.

## MATERIALS AND METHODS

**Materials and reagents:** Cucumber, red pepper, garlic, ginger and salt used for cucumber pickling were purchased from a nearby farmer's market.

Acids standards were obtained from different suppliers. Citric acid (99.0%), succinic acid (99.0%), malic acid (99.0%) and tartaric acid (99.0%) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Oxalic acid (99.0%) was obtained from Shanghai Meixin Ltd. Acetic acid (99.0%) and lactic acid (99.0%) was obtained from Sinopharm Chemical Reagent (China). Methanol (HPLC-grade) was obtained from Si You Ltd (Tianjin, China). Ultra-pure water was obtained from a pure water instrument (Ultrapure, Chengdu).

LABs i.e., *Lactobacillus acidophilus* ZJU10023 and *Lactobacillus helveticus* ZJU10036 were stored in our laboratory. They were incubated aerobically onto fresh MRS broth for 24 h at 37°C to be activated before the experiments. Then they were incubated with a proportion of 1:1 as the starter.

**Pickled cucumber preparation:** Kimchi samples for analysis were prepared according to the method described by Buscher *et al.* (2011). Freshly harvested and undamaged cucumbers were properly washed and cut into small cubes (approximately 2×7 cm). These cubes were blanched in boiling water for 1 min at 70°C. Then every 200 g of these cubes were dispensed in six 500 mL jars. Based on the pre-results of response surface methodology, brine solution of 3.07% table salt, 1% seasoning (red pepper, minced garlic, ginger) and 1.39% sucrose was prepared. Then 400 mL of the prepared brine solution was added to each bottle. Among these six bottles, three were fermented directly as SF. Others were inoculated with 3.81% (w/v) (according to pre-results) prepared starter as LABIF. The fermentation processes were carried on at room temperature.

**Texture analysis:** The texture of pickled cucumber was quantified using a texture analyzer. Since they were severely bloated in some treatments (Fleming *et al.*, 1995), cucumbers were cut longitudinally into halves and placed on the test plate. A 0.5 cm diameter tip was used to measure the firmness of the sample and expressed as kg force. For each sample, 10 measurements were conducted and the average of measured values was calculated.

**Microscopic observation of pickled cucumber tissue:** Microscopic observation was conducted to reflect textural quality of the pickled cucumbers. Samples were treated as described by Yoo *et al.* (2006). The pickled cucumber tissue (1×1 cm) around the midrib was cross-sectioned, freeze-dried and coated with gold using an Ion Sputtering Device. Microscopic observation of vascular bundle tissue was performed by using a Scanning Electron Microscope (SEM).

**Sensory evaluation:** Sensory evaluation of pickled cucumber was carried out with 20 trained panel members. Descriptive characteristics (appearance,

flavour, palate, texture) were subjectively evaluated using grades from 1 (the least preferred) to 5 (the most preferred). A different weighting coefficient was distributed to every indicator, namely appearance (0.2), flavor (0.25), palate (0.25) and texture (0.3). The final score was calculated according to the weighting method Eq. (1). Panelists were asked to wash their mouths with water after testing each sample:

$$\text{Final score} = a1 \times 0.2 + a2 \times 0.25 + a3 \times 0.25 + a4 \times 0.3 \quad (1)$$

a1, grade of appearance, a2, grade of flavor, a3, grade of palate, a4, grade of texture.

**Organic acid analysis:** The analyses of organic acids were carried out on a HPLC system (Shimadzu, Japan) equipped with a UV-VIS multi wavelength detector. A HPLC column (C<sub>18</sub>, 250×4.6 mm, 5 μm particle size, Sun Fire) was used. Chromatographic conditions were: the mobile phase was phosphate buffer 0.01 mol/L-3.0% methanol, pH 2.8, detection at 210 nm of Ultraviolet (UV) absorbance, 0.8 mL/min flow rate and 10 μL injection volume. The column temperature was kept ambient. The mobile phase was prepared and filtered through a 0.22 μm nylon filter membrane (Jin Long) before use.

Fermented cucumber juice for HPLC analysis was taken every two days till the seventh day to determine the changes of organic acids. Before injection, samples were centrifuged at 3000 g for 15 min. The supernatant obtained was filtered through a 0.22 μm filter and injected into HPLC system.

**Delineation of calibration curves:** To delineate the calibration curves, standard solution of mixed organic acids were prepared. Initial concentration of seven acids

were tartaric acid 1, oxalic acid 2, malic acid 1, citric acid 1, succinic acid 2, lactic acid 2 and acetic acid 1 g/L, respectively. Then the solution was diluted 5 times and five different concentrations were finally obtained. Before injected into HPLC system for testing, these solutions were filtered through a 0.22 μm filter.

**Liquid chromatographic analysis:** Chromatographic peaks were identified by comparison of elution order and retention times with those of standards. The quantification of the studied compounds was carried out using the external standard method. Final values were shown in the form of means±SD (n = 3).

## RESULTS AND DISCUSSION

**Firmness and sensory score:** The firmness and sensory evaluation of cucumbers was shown in Table 1 and 2. The firmness of cucumbers decreased after fermentation. Compared to fresh cucumber, the firmness underwent a decrease of 11.65 and 2.37% respectively in SF and LABIF. That was coincident with sensory evaluation scores, which was 3.6 and 3.85

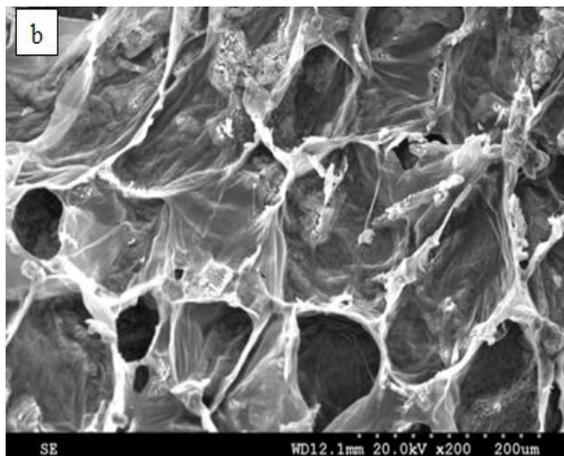
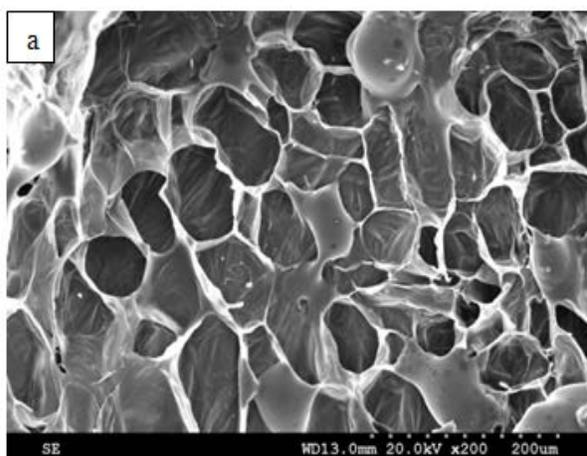
Table 1: Firmness of cucumbers with different treatments

Number	Fresh cucumber	SF cucumber	LABIF cucumber
1	1913.90 <sup>a</sup>	1689.70	1857.20
2	1901.80	1670.60	1856.80
3	1898.60	1668.00	1856.40
4	1892.70	1664.70	1849.00
5	1870.20	1658.50	1833.60
6	1858.80	1657.60	1829.40
7	1853.10	1647.10	1812.40
8	1834.90	1611.50	1763.40
9	1799.40	1596.60	1756.80
10	1788.70	1579.30	1756.60
Means	1861.21	1644.36	1817.16

<sup>a</sup>: firmness of cucumbers, with a unit of kg

Table 2: Sensory evaluation of pickled cucumber

Treatment	Appearance/0.2	Flavour/0.25	Palate/0.25	Texture /0.3	Final score
SF (score)	3.7±0.700	3.7±0.78	3.70±0.77	3.6±0.60	3.49±0.38
LABIF (score)	3.85±0.83	3.8±0.73	3.65±0.64	3.85±0.73	3.79±0.3



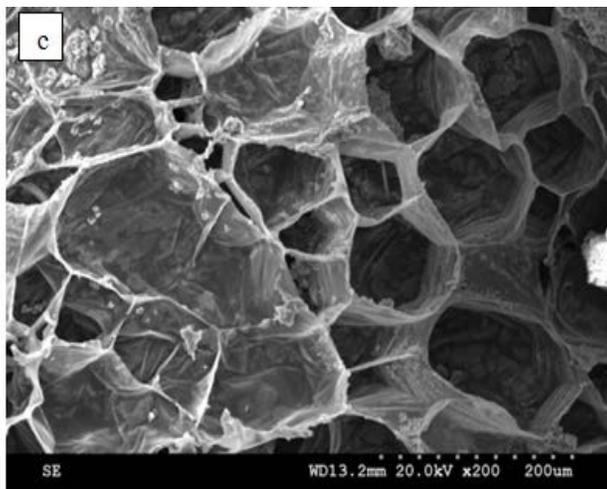


Fig. 1: Scanning electron microscope photographs of vascular bundle tissue of (a) fresh cucumber, (b) SF cucumber, (c) LABIF cucumber. Magnification scale is 200×

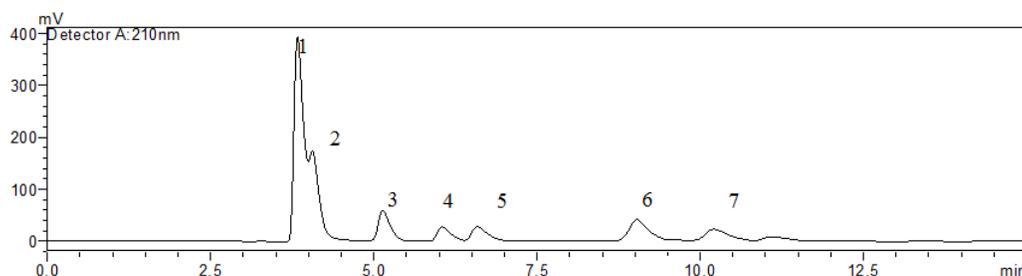


Fig. 2: Calibration HPLC chromatogram for organic acids- 1, oxalic acid; 2, tartaric acid; 3, malic acid; 4, lactic acid; 5, acetic acid; 6, citric acid; 7, succinic acid

regarding to the texture of SF cucumber and LABIF cucumber, respectively. Furthermore, the final score of LABIF cucumber was obviously higher than that of SF cucumber.

**SEM photographs:** Figure 1 shows the photographs of vascular bundle tissue of pickled cucumber after 7 days fermentation. Texture of fermented food is influenced by structural changes (Yoo *et al.*, 2006). In fresh cucumber, parenchyma cells had oval shape (a) and with the process of salting and fermentation, cells were expanded and middle lamella was weakened (b, c). However, LABIF cucumbers (c) still kept most of the original structure and that was the reason for higher firmness in texture analysis (Table 1).

**Calibration chromatogram and parameters for organic acids:** A chromatogram of seven organic acids was shown in Fig. 2 and Table 3 shows their retention times and linear equations. As shown in the chromatogram, oxalic acid had some interference with tartaric acid because of similar retention time, which did not influence the identification of them. Furthermore, as the values of  $R^2$  of seven organic acids

were all higher than 0.9994, there was comparative reliability of the linear equations.

**Changes of organic acids in SF:** Changes of seven organic acids in SF can be seen from Fig. 3 and Table 4. As was shown, most organic acids increased during seven days' fermentation. Lactic acid increased fastest, from 0.24 to 3.772 mg/mL. Following was acetic acid, increased from 0.207 to 3.206 mg/mL. Oxalic acid hardly increased during seven days fermentation. On the first day, citric acid was not detected, maybe due to too little content or the existence of other interferences.

**Changes of organic acids in LABIF:** During the process of LABIF, the variation of organic acids was similar to that of SF while the contents differed (Fig. 4). Most organic acids underwent an increase during the fermentation of seven days, except for tartaric acid decreased on the third day and acetic acid decreased on the third day but then increased on the fifth day. Throughout the fermentation, lactic acid was the maximum organic acid. Oxalic acid remained stable through the whole process. Citric acid was not detected

Table 3: Relative parameters of organic acids

Organic Acid	Retention time (min)	Linear equation	Related coefficient (R <sup>2</sup> )
Oxalic acid	3.827	y = 8E+06x-16939	R <sup>2</sup> = 0.9995
Tartaric acid	4.054	y = 7E-07x-0.0018	R <sup>2</sup> = 0.9997
Malic acid	5.131	y = 1E-06x-0.0015	R <sup>2</sup> = 0.9999
Lactic acid	6.041	y = 2E-06x-0.0032	R <sup>2</sup> = 0.9997
Acetic acid	6.579	y = 2E-06x+0.0067	R <sup>2</sup> = 0.9994
Citric acid	9.023	y = 1E-06x-0.0010	R <sup>2</sup> = 0.9999
Succinic acid	10.199	y = 2E-06x-0.0022	R <sup>2</sup> = 0.9997

Table 4: Contents of organic acids in pickled cucumber mg/mL

Fermentation way	Time/d	Oxalic acid	Tartaric acid	Malic acid	Lactic acid	Acetic acid	Citric acid	Succinic acid
LABIF	1	0.012±0.00	1.016±0.29	0.005±0.01	1.365±0.15	0.763±0.07	0.195±0.04	0.394±0.09
	3	0.025±0.01	0.849±0.08	0.008±0.01	3.215±0.22	0.407±0.13	—	0.299±0.05
	5	0.030±0.01	1.129±0.08	0.055±0.03	4.526±0.52	0.470±0.11	1.115±0.04	0.735±0.42
	7	0.029±0.01	1.354±0.04	0.434±0.04	4.942±0.10	4.034±0.21	0.874±0.21	0.765±0.21
SF	1	0.042±0.00	0.164±0.01	0.213±0.01	0.240±0.03	0.207±0.02	—	0.254±0.01
	3	0.033±0.01	0.293±0.11	0.138±0.12	1.284±0.19	0.842±0.19	0.018±0.02	0.282±0.03
	5	0.054±0.00	0.452±0.12	0.546±0.09	3.380±0.22	3.270±0.01	0.417±0.31	0.066±0.21
	7	0.027±0.01	0.537±0.11	0.101±0.01	3.772±0.11	3.206±0.30	0.863±0.08	0.732±0.11

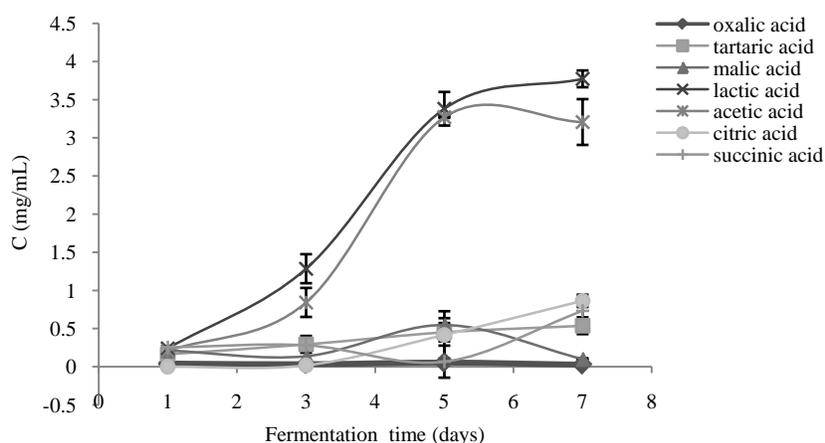


Fig. 3: Changes of organic acids in SF

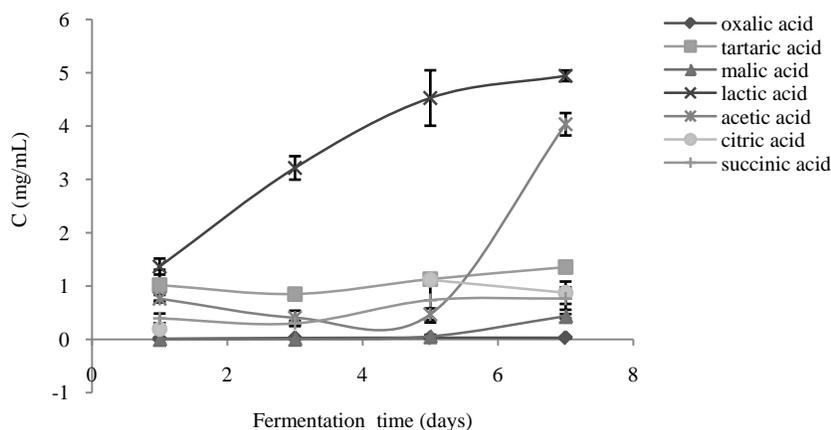


Fig. 4: Changes of organic acids in LABIF

on the second day probably because of the interference of other substance.

However, it was not expected that acetic acid reached 4.034 mg/mL on the seventh day and was higher than that of SF (3.206 mg/mL), whether it was

due to too much acetic acid by LAB need more elucidation.

**Contrast of two different fermentation ways:** The organic acid chromatogram (Fig. 5) of SF and LABIF

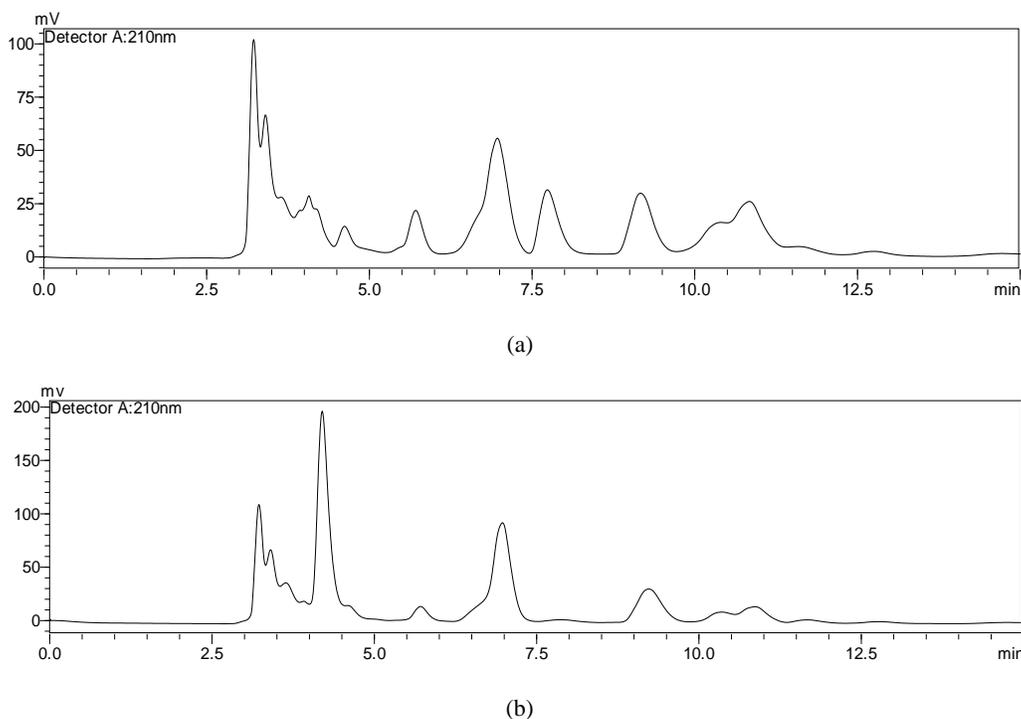


Fig. 5: Chromatograms of organic acids during SF (a) and LABIF (b)

cucumbers in the seventh day indicated that SF produced more kinds of organic acids while all the seven organic acids investigated existed in both cucumbers. The specific values can be achieved from Table 4.

In both cucumbers, lactic acid showed the highest concentration among all the organic acids detected, followed by acetic acid and tartaric acid. Others were detected at lower levels.

However, mean content of all the organic acids in LABIF were higher than that of SF. For instance, lactic acid and tartaric acid reached 4.942 and 1.354 mg/mL, respectively in LABIF, while it was only 3.772 and 0.537 mg/mL in SF. Besides, malic acid in LABIF was 3.3 times higher than that of SF. The results implied LABIF a good way to increase the content of organic acids. In addition, sourness of tartaric acid was stronger than that of malic acid and citric acid and that is why the taste and flavor of LABIF cucumber was stronger than that of SF cucumber.

However, it was not expected that acetic acid accumulation in LABIF lagged behind that in SF, while reached 4.034 mg/mL on the seventh day and was higher than that of SF (3.206 mg/mL). Based on the distinctiveness of Fig. 3 and 4, besides the organic acid quantity, the most significant distinctiveness was that in SF (Fig. 3) lactic acid and acetic acid accumulated simultaneously, while in LABIF (Fig. 4) the accumulation of acetic acid lagged behind that of lactic acid in 5 days but increased sharply in last 2 days when lactic acid had accumulated in a high concentration. Based on the organic acid accumulation pattern, it could be deduced that in SF LAB and Acetic Acid

Bacteria (AAB) fermented simultaneously and neither being in dominant position, which was unbeneficial to inhibit spoilage microorganisms. While in LABIF, LAB fermented beforehand thus being in dominant position, then AAB fermented vigorously in an acidic condition created by LAB, which was beneficial to inhibit spoilage microorganisms. In our pre-results, the total viable count and spoilage microorganism in SF were much higher than that in LABIF. High concentrations of lactic acid and acetic acid were beneficial to the flavor and storage of cucumbers (Franco and Pérez-Díaz, 2012; Franco *et al.*, 2012). As we can see, the different way of acetic acid accumulation was the distinctive feature between LABIF and SF which led to different flavor and quality of the two cucumbers.

## CONCLUSION

The characteristics of pickled cucumber were evaluated in this study. Contrast of two different fermentation ways from the point of macro and micro was conducted. As the results, LABIF shows more advantage than SF regarding to the taste, firmness, vascular bundle tissue, composition and contents of organic acids of pickled cucumber and the acetic acid accumulation pattern could be regarded as the distinctive feature between SF and LABIF.

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