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Vegetables and Yoghurt Fermentation of Lactobacillus Strains (การหมักผักและโยเกิร์ตของสายพันธุ์แล็กโตบาซิลลัส)

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การหมักผักและโยเกิร์ตของสายพันธุ์แล็กโตบาซิลลัส

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บทคัดย่อ

การหมักผักกาดเขียวปลี และ ผักรวมโดยใช้สตาร์ทเตอร์ของเชื้อ *Lactobacillus* sp. L2-1 , *L. farciminis* 73-1 และ , *L. plantarum* P 7-1 เปรียบเทียบกับการหมักตามธรรมชาติ โดยแปรปริมาณเกลือเป็นร้อยละ 1 -2 และปริมาณ น้ำตาลเป็นร้อยละ 2-4 พบว่าการใช้สตาร์ทเตอร์หมักผักกาดเขียวปลีเป็นเวลา 3 วัน มีค่าพีเอชเป็น 2.60 - 2.75 และมีปริมาณของกรดแล็กติก 2.30-3.09 มก/มล. ขณะที่การหมักตามธรรมชาติ มีค่าพีเอชเป็น 2.82 - 3.22 และมีปริมาณกรดแล็กติก 1.63 - 2.36 มก/มล. สำหรับ การหมักผักรวม โดยใช้สตาร์ทเตอร์หมักเป็นเวลา 3 วัน ค่าพีเอชเป็น 2.80 - 2.93 และมีปริมาณของกรดแล็กติก 2.02 - 2.32 มก/มล. ขณะที่การหมักตามธรรมชาติจะมีค่าพีเอช 2.96-3.25 และมีปริมาณของกรดแล็กติก 1.69-2.27 มก/มล. การวิเคราะห์ข้อมูลทางสถิติของการหมักผักกาดเขียวปลี และ ผักรวม ปริมาณเกลือ น้ำตาลและ ชนิดของเชื้อมีผลต่อค่าพีเอชและปริมาณกรดแล็กติกอย่างมีนัยสำคัญทางสถิติ ($p \leq 0.05$) การผลิตโยเกิร์ตโดยใช้เชื้อ L2-1 และ 73-1 ที่มีการแปรปริมาณน้ำตาลเป็นร้อยละ 6 , 8 และ 10 บ่มที่ 37°ซ. นาน 24 และ 48 ชม. เปรียบเทียบกับการหมักโยเกิร์ตด้วย โยเกิร์ตสตาร์ทเตอร์ผงของเชื้อ *Lactobacillus bulgaricus* และ *Streptococcus thermophilus* บ่มที่ 45°ซ. พบว่า เชื้อ L2 - 1สามารถผลิตโยเกิร์ตที่มีคุณภาพดีกว่าเชื้อ 73 - 1 ที่ความเข้มข้นของน้ำตาลร้อยละ 10 ซึ่งบ่มที่ 37°ซ. นาน 24 ชม. และใกล้เคียงกับโยเกิรต์สตาร์ทเตอร์ โยเกิรต์ที่ได้มีค่าพีเอชเป็น 3.62 , 3.75 และ 3.70 ตามลำดับ มีปริมาณของกรดแล็กติก 0.82 , 0.60 และ 0.92 มก/มล. และมีค่าความหนืด 1203.11 , 675.07 และ 1344.51 มิลลิพาสคาล ตามลำดับ การวิเคราะห์ข้อมูลทางสถิติพบว่าปริมาณน้ำตาล และชนิดของเชื้อมีผลต่อค่าพีเอช ปริมาณของกรดแล็กติก และค่าความหนืดอย่างมีนัยสำคัญทางสถิติ ($p \leq 0.05$)

กุญแจคำ

แล็กติกแอสิดแบคทีเรีย แล็กโตบาซิลลัส การหมักผัก โยเกิร์ต

Original Article

Vegetables and Yoghurt Fermentation of *Lactobacillus* Strains

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Abstract

Lactobacillus sp. L2-1, *Lactobacillus farciminis* 73-1, and *Lactobacillus plantarum* P7-1 were used for fermenting green mustard and mixed vegetables in comparison to natural fermentation. The salt and sugar concentration were varied to 1-2% and 2-4%, respectively. The results indicated that fermenting green mustard for three days brought the pH down to 2.60-2.75, and contained 2.30-3.09 mg/ml lactic acid while the natural fermentation had a pH of 2.82-3.22, and contained 1.63-2.36 mg/ml lactic acid. As for mixed vegetables, the three days of fermentation gave a pH of 2.80-2.93, and contained 2.02-2.32 mg/ml lactic acid ; while natural fermentation had a pH of 2.96-3.25 and contained 1.69-2.27 mg/ml lactic acid. Statistical analysis of green mustard and mixed vegetables showed that the contents of salt, sugar and starter cultures had a significant effect on the pH and the amount of lactic acid ($p \leq 0.05$). The production of yoghurt by L2-1 and 73-1 using sugar concentration of 6, 8 and 10%, incubated at 37°C for 24 and 48 hours were compared to powder yoghurt starter (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) which was incubated at 45°C. With 10% sugar concentration at 37°C for 24 hours, the quality of yoghurt produced by L2-1 was better than that of 73-1 and was the same as using yoghurt starter. The pHs of products were 3.62, 3.75 and 3.70 and the concentration of lactic acid was 0.82, 0.60 and 0.92 mg/ml, and viscosities were 1203.11, 675.07 and 1344.51 mpas, respectively. Statistical analysis indicated that the pH, lactic acid concentration and the viscosity were affected by the content of sugar and the strain of starter cultures ($p \leq 0.05$).

Key words

Lactic acid bacteria, *Lactobacillus*, Vegetable fermentation, Yoghurt

Introduction

Lactic acid bacteria (LAB) have been responsible for the fermentative preservation of many foods, including fruits, vegetables, and milk (1-2). The fermentation increases the availability of seasonal vegetables and produces a desired sensory product quality (3-4). Since lactic acid bacteria are normally present on living plants (5), spontaneous lactic acid fermentation occurs when vegetables are brined. Starter cultures may be used to control the process in order to prevent spoilage and to obtain products of a consistently high and desired quality (6). The quality of strains used in starters is particularly important (7). The object of this study was to investigate the

fermentation that would potentially provide a simple method for the preservation of green mustard and mixed vegetables, and fermentation yoghurt. We described the effects of pure cultures comprising homofermentative, L-(+) lactic acid producing *Lactobacillus* strains which brought about rapid fermentation of various raw materials.

Materials and Methods

Bacteria and cultivation. *Lactobacillus plantarum* P7-1 isolated from pickled mustard (8), *Lactobacillus* sp. L2-1 from bagasse (9) and *L. farciminis* 73-1 from pla-ra (10) were used in this study. They were cultivated in glucose-yeast extract-peptone-beef extract (GYPB) medium or De Man, Rogosa and Sharpe (MRS) medium (10-11). Tomato-potato-glucose broth composed of 100 ml tomato juice (extracted from 60 g tomato in 100 ml water), 25 ml potato juice (extracted from 80 g potato in 100 ml water) and 1.0 g glucose, and adjusted to pH 6.8 was prepared for cultivation of liquid starter cultures. They were incubated at 30°C, except for the investigation of effects of temperature.

Determination of characteristics. The strains were characterized phenotypically as previously described (8,10,12). Diacetyl formation was tested by the method of Phalip et al. (13). Peptidoglycan was determined as described by Komagata and Suzuki (14). Isomer of lactic acid was analyzed by the method of Okada et al. (15). Antibacterial activity against the growth of *S. aureus* ATCC 25923 and *B. subtilis* ATCC 6633 was examined by the method of Samelis and Metaxopoulous (16).

Fermentation of vegetables. Green mustard (1.0 kg.) or mixed vegetables (cabbage 0.6 kg., carrot 0.2 kg., ginger 0.1 kg. and chilli 0.05 kg.) were washed throughly in clear water, and then were peeled and cut into uniform pieces. Mature cabbages were trimmed to remove the outer broken or dirty leaves. Then the cores were cut by a reversing cover that left the cut core in the head. These pieces were blanched in boiled water for 5 minutes. The liquid starter culture (2% v/v) in brine was poured over the pieces of vegetables. The controlled brine with no starter culture for each type of vegetables was prepared similarly. In this fermentation, salt and sugar were varied to 1 and 2% and 2 and 4%, respectively. Four batches with triplicates were carried out for each culture. At the time of filling to the desired level the shreds were covered with a sheet of plastic large enough to cover the area on the top of the plastic box. The vegetables were fermented for 3 days at 30°C, after that the aliquots of brine were taken and analyzed for pH and acidity as described by Meade and Chen (17). Categorical data were analyzed using asymmetrical Factorial Analysis Test 4 x 2 x 2 analyzing the statistical difference by using DMRT (Duncan's Multiple Range Test)(18). The cell numbers of lactic acid bacteria were counted on GYPB-$CaCO_3$ agar by diluting the sample to 1:10 and more (6-12), and each diluted samples was put in the petridish, then mixed with the medium using the pour plate techniques (19).

Fermentation of yoghurt. *Lactobacillus* sp. L2-1 and *L. farciminis* 73-1 were selected in producing yoghurt compared with mixed powder yoghurt starter (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*). Pasteurized milk with 6, 8 or 10% sugar was treated at 90°C for 5 min. Batches of yoghurt were fermented with 5% starter and incubated at 37°C for 24 to 48 h. Other batches produced by mixed yoghurt starter (0.01%) were incubated at 45°C for 24 to 48 h. Three batches with triplicates were done for each strain. The yoghurt was incubated for 24 to 48 h, then an aliquot of yoghurt was taken and analyzed by the same method used in the fermentation of pickled vegetables. The pH, acidity, viscosity and the cell numbers of lactic acid bacteria of assessed yoghurt were determined. In the preparation of drinking yoghurt, the natural yoghurt was diluted with water (1 : 0.3 v/v) and added with 5% sugar and 0.05% pectin. The sensory quality of the resulting products was evaluated (20). Categorical data were analyzed using asymmetrical Factorial Analysis Test 3 x 3 x 1 to analyze the statistical difference by using DMRT (Duncan's Multiple Range Test) (18).

Result and Discussion

Determination of characteristics. The characteristics of *Lactobacillus plantarum* P7-1, *Lactobacillus* sp. L2-1 and *L. farciminis* 73-1 are shown in Table 1. These bacteria showed negative reactions for catalase,

Table 1. Characteristics of *Lactobacillus* strains.

Characteristic		L2-1	73-1	P7-1
Cell form		Rods	Rods	Rods
Cell size (μm)		0.5 –1.0 x 1.0 –5.0	0.5 –1.0 x 1.0 –5.0	0.5 –1.0 x 1.0 –5.0
Cell arrangement		singly, in pairs or in chains	singly, in pairs or in chains	singly, in pairs or in chains
Catalase		-	-	-
Esculin hydrolysis		+	+	+
Gelatin hydrolysis		-	-	-
Nitrate reduction		-	-	-
Diacetyl formation		+	+	-
Growth at	10 °C	-	-	-
	30 °C	+	+	+
	40 °C	+	+	+
	pH 3.0	+	+	+
	pH 8.0	+	+	+
Growth in	4.0% NaCl	+	+	+
	5.0% NaCl	-	+	+
	6.0% NaCl	-	+	+
	7.0% NaCl	-	+	-
	8.0% NaCl	-	+	-
Acid from:				
	L-Arabinose	+	+	+
	D-Cellobiose	+	+	+
	D-Fructose	+	+	+
	D-Galactose	+	+	+
	Lactose	+	+	+
	D-Maltose	+	+	+
	D-Melibiose	+	+	+
	L-Raffinose	-	+	-
	D-Ribose	+	+	+
	Sucrose	+	+	+
	Starch	-	-	-
	D-Xylose	+	+	+
Diaminopimelic acid in cell wall		-	-	+
Isomer of lactic acid		L	L	DL
Inhibition of				
Staphylococcus aureus ATCC 25923		+	+	-
Bacillus subtilis ATCC 6633		-	+	-

+, positive reaction; -, negative reaction.

nitrate reduction, hydrolysis of gelatin, and growth at 10°C, but gave positive results to growth at 30-40°C , growth at pH of 3 to 9 as well as growth in GYPB broth containing 1 and 4 % NaCl (73-1 could grow at 8% NaCl). The strains L2-1 and 73-1 produced L-(+)lactic acid homofermentatively while strain P7-1 produced DL-lactic acid. L2-1 could inhibit *Staphylococcus aureus* ATCC 25923, whereas the strain 73-1 could inhibit *S. aureus* ATCC 25923 and *Bacillus subtilis* ATCC 6633, compared with that of nisin (1 : 50) (data not shown). The P7-1 strain contained *meso*-diaminopimelic acid in its cell wall, but the L2-1 and 73-1 did not.

Fermentation of vegetables. The vegetables were fermented for 3, 7 and 14 days at 30°C . In comparison to L2-1 and 73-1, fermenting the green mustard with P7-1 brought the pH down and produced lactic acid at a greater margin. Pure culture fermentation was compared with natural fermentation. The result also indicated that fermenting green mustard with liquid cultures for

Table 2. Salt and sugar concentrations, pH, lactic acid, and cell number in fermenting green mustard for 3 days.

Strain	Salt (%)	Sugar (%)	Mean ± SD		
			pH	Lactic acid (mg/ml)	LAB (cells/ml)^{NS}
Natural	1	2	$3.22^{h} \pm 0.01$	$1.63^{f} \pm 0.10$	$3.19x10^{10} \pm 3.1x10^{9}$
P7-1			$2.60^{bcd} \pm 0.07$	$2.75^{abc} \pm 0.03$	$1.19x10^{10} \pm 1.19x10^{9}$
L2-1			$2.72^{de} \pm 0.06$	$2.30^{e} \pm 0.03$	$4.75x10^{8} \pm 2.76x10^{7}$
73-1			$2.75^{de} \pm 0.07$	$2.42^{e} \pm 0.03$	$1.18x10^{10} \pm 1.9 x10^{9}$
Natural	1	4	$2.87^{f} \pm 0.02$	$2.23^{e} \pm 0.00$	$2.95x10^{12} \pm 3.53x10^{10}$
P7-1			$2.50^{a} \pm 0.00$	$3.09^{a} \pm 0.10$	$3.9 x10^{11} \pm 2.68x10^{8}$
L2-1			$2.62^{bc} \pm 0.02$	$2.71^{abc} \pm 0.03$	$8.5 x10^{9} \pm 3.5 x10^{6}$
73-1			$2.65^{bcd} \pm 0.00$	$2.76^{abc} \pm 0.03$	$4.60x10^{10} \pm 3.2 x10^{9}$
Natural	2	2	$3.08^{g} \pm 0.11$	$1.91^{e} \pm 0.04$	$2 x10^{10} \pm 7.07x10^{8}$
P7-1			$2.60^{bc} \pm 0.02$	$3.03^{ab} \pm 0.07$	$2.9 x10^{9} \pm 1.41x10^{9}$
L2-1			$2.65^{c} \pm 0.06$	$2.64^{cd} \pm 0.03$	$1.5 x10^{10} \pm 7.78x10^{8}$
73-1			$2.67^{cd} \pm 0.01$	$2.70^{bc} \pm 0.00$	$3.0 x10^{12} \pm 1.97x10^{8}$
Natural	2	4	$2.82^{ef} \pm 0.02$	$2.36^{de} \pm 0.07$	$5.3 x10^{11} \pm 7.3 x10^{10}$
P7-1			$2.55^{ab} \pm 0.02$	$2.99^{abc} \pm 0.09$	$1.05x10^{12} \pm 4.9 x10^{8}$
L2-1			$2.61^{bc} \pm 0.01$	$2.86^{abc} \pm 0.03$	$3.0x10^{8} \pm 2.1 x10^{6}$
73-1			$2.65^{bc} \pm 0.01$	$3.09^{a} \pm 0.03$	$2.4x10^{10} \pm 1.5 x10^{9}$

a, b, c... denote the significant differences ($p < 0.05$) in the same column.
NS denotes no significant differences ($p > 0.05$).

three days brought the pH down to 2.6 -2.75, and contained lactic acid 2.30-3.09 mg/ml while the natural fermentation had pH 2.82-3.22 and contained 1.63-2.36 mg/ml lactic acid (Table 2). The three day fermentation of mixed vegetables had pH 2.80-2.93 and contained 2.02-2.32 mg/ml lactic acid while natural fermentation had pH 2.96-3.25 and contained 1.69-2.27 mg/ml lactic acid (Table 3). The pH decreased by P7-1 was more than those caused by L2-1 and 73-1 in the fermentation of mixed vegetables. L2-1 strain could produce more lactic acid, compared to P7-1 and 73-1. Statistical analysis of both fermentations indicated that the contents of salt, sugar and starter cultures had significant effect on the pH and amount of lactic acid ($p \leq 0.05$) , but had no effect on the cell number of lactic acid bacteria ($p > 0.05$). On the seventh day of the fermentation, some of the naturally fermented green mustard and mixed vegetable samples began to have the presence of yeasts (4). All the vegetables retained their natural colour and were found to be acceptable with respect to appearance, flavour, crispness, sourness, and taste when presented to the taste panel (descriptive). They could also be kept for a longer period comparing to those fermented naturally (3).

Fermentation of yoghurt. From the experiment, L2-1 was suitable for the production of yoghurt while

Table 3. Salt and sugar concentration, pH, lactic acid, and cell number in fermenting mixed vegetables for 3 days.

Strain	Salt (%)	Sugar (%)	Mean ± SD		
			pH	Lactic acid(mg/ml)	LAB (cells/ml)^{NS}
Natural	1	2	$3.25^{c} \pm 0.77$	$1.78^{e} \pm 0.07$	$3.9x10^{10} \pm 1.48x10^{9}$
P7-1			$2.80^{a} \pm 0.42$	$2.25^{c} \pm 0.03$	$5.0x10^{9} \pm 0.00$
L2-1			$2.90^{ab} \pm 0.02$	$2.02^{de} \pm 0.17$	$3.0\ x10^{10} \pm 3.5\ x10^{6}$
73-1			$2.93^{ab} \pm 0.07$	$2.92^{c} \pm 0.03$	$2.95x10^{9} \pm 1.9\ x10^{6}$
Natural	1	4	$2.96^{ab} \pm 0.02$	$2.27^{d} \pm 0.03$	$4.9\ x10^{11} \pm 3.11x10^{9}$
P7-1			$2.80^{a} \pm 0.03$	$3.15^{c} \pm 0.07$	$5.0\ x10^{10} \pm 1.76x10^{8}$
L2-1			$2.87^{a} \pm 0.06$	$3.33^{a} \pm 0.07$	$2.9\ x10^{11} \pm 2.13x10^{9}$
73-1			$2.90^{ab} \pm 0.02$	$2.92^{c} \pm 0.03$	$2.85x10^{9} \pm 1.87x10^{8}$
Natural	2	2	$3.11^{c} \pm 0.07$	$1.69^{e} \pm 0.02$	$1.0\ x10^{10} \pm 3.53x10^{8}$
P7-1			$2.86^{a} \pm 0.04$	$3.12^{c} \pm 0.02$	$1.95x10^{12} \pm 1.34x10^{9}$
L2-1			$2.88^{ab} \pm 0.09$	$3.26^{b} \pm 0.03$	$1.20x10^{11} \pm 5.65x10^{9}$
73-1			$2.92^{ab} \pm 0.07$	$2.84^{d} \pm 0.65$	$8.5x10^{9} \pm 5.9\ x10^{8}$
Natural	2	4	$3.10^{c} \pm 0.07$	$2.02^{de} \pm 0.07$	$2.05x10^{13} \pm 7.07x10^{10}$
P7-1			$2.85^{a} \pm 0.10$	$2.92^{c} \pm 0.00$	$2.10x10^{10} \pm 1.05x10^{9}$
L2-1			$2.88^{ab} \pm 0.00$	$3.24^{b} \pm 0.02$	$1.9x10^{10} \pm 3.53x10^{8}$
73-1			$2.92^{ab} \pm 0.09$	$2.81^{d} \pm 0.00$	$1.8x10^{9} \pm 7.78x10^{8}$

$^{a, b, c, \ldots}$ denote the significant differences ($p < 0.05$) in the same column.
^{NS} denotes no significant differences ($p > 0.05$).

Table 4. Sugar concentrations, pH, lactic acid, viscosity, and cell number in fermenting yoghurt for 24 h.

Strain	Sugar (%)	pH	Lactic acid (mg/ml)	Viscosity (mpas)	LAB (cells/ml)^{NS}
Yoghurt starter	6	$4.05^{b} \pm 0.07$	$0.47^{cd} \pm 0.35$	$611.44^{d} \pm 0.69$	$1.2x10^{9} \pm 1.0\ x10^{8}$
L2-1		$3.93^{b} \pm 0.02$	$0.45^{cd} \pm 0.14$	$604.05^{d} \pm 6.23$	$2.5x10^{9} \pm 1.0\ x10^{6}$
73-1		$4.10^{bc} \pm 0.14$	$0.42^{d} \pm 0.35$	$421.78^{f} \pm 11.62$	$5.0x10^{10} \pm 2.5\ x10^{6}$
Yoghurt starter	8	$3.77^{ab} \pm 0.03$	$0.77^{b} \pm 0.03$	$1115.42^{b} \pm 12.78$	$1.0x10^{10} \pm 1.5\ x10^{8}$
L2-1		$3.80^{b} \pm 0.00$	$0.72^{b} \pm 0.03$	$950.83^{c} \pm 20.03$	$1.2x10^{11} \pm 1.0x10^{8}$
73-1		$3.83^{b} \pm 0.03$	$0.50^{cd} \pm 0.00$	$559.80^{d} \pm 7.89$	$1.2x10^{11} \pm 6.5\ x10^{9}$
Yoghurt starter	10	$3.70^{a} \pm 0.21$	$0.92^{a} \pm 0.70$	$1344.51^{a} \pm 6.63$	$1.2x10^{9} \pm 5.0\ x10^{8}$
L2-1		$3.62^{a} \pm 0.17$	$0.82^{a} \pm 0.35$	$1203.11^{b} \pm 3.55$	$2.5x10^{11} \pm 6.7\ x10^{8}$
73-1		$3.75^{b} \pm 0.07$	$0.60^{c} \pm 0.07$	$675.07^{d} \pm 0.56$	$3.5x10^{10} \pm 5.0\ x10^{9}$

$^{a, b, c, \ldots}$ denote the significant differences ($p < 0.05$) in the same column.
^{NS} denotes no significant differences ($p > 0.05$).

Table 5. Sugar concentrations, pH, lactic acid, viscosity, and cell number in fermenting yoghurt for 48 h.

Strain	Sugar (%)	pH	Lactic acid (mg/ml)	Viscosity (mpas)	LAB (cells/ml)^{NS}
Yoghurt starter	6	$3.25^{b} \pm 0.07$	$1.05^{c} \pm 0.07$	$910.13^{e} \pm 0.18$	$3.5x10^{9} \pm 1.0x10^{8}$
L2-1		$3.26^{b} \pm 0.84$	$1.07^{c} \pm 0.28$	$707.21^{g} \pm 6.05$	$2.9x10^{10} \pm 6x10^{9}$
73-1		$3.53^{c} \pm 0.04$	$0.84^{d} \pm 0.00$	$589.78^{h} \pm 1.10$	$3.2x10^{11} \pm 2.5x10^{5}$
Yoghurt starter	8	$3.24^{ab} \pm 0.07$	$1.17^{ab} \pm 0.03$	$1649.08^{b} \pm 12.78$	$1.4x10^{10} \pm 5.0x10^{8}$
L2-1		$3.28^{b} \pm 0.00$	$0.75^{c} \pm 0.13$	$1072.50^{d} \pm 20.0$	$5.5x10^{10} \pm 6.5x10^{6}$
73-1		$3.27^{b} \pm 0.10$	$0.90^{d} \pm 0.00$	$724.50^{g} \pm 7.89$	$3.5x10^{9} \pm 1.9x10^{8}$
Yoghurt starter	10	$3.22^{a} \pm 0.03$	$1.23^{a} \pm 0.70$	$1820.50^{a} \pm 0.70$	$1.2x10^{10} \pm 7.0x10^{8}$
L2-1		$3.22^{a} \pm 0.14$	$1.12^{b} \pm 0.35$	$1372.0^{c} \pm 19.79$	$2.5x10^{10} \pm 6.7x10^{8}$
73-1		$3.29^{b} \pm 0.00$	$1.09^{b} \pm 0.04$	$805.07^{f} \pm 6.96$	$5.5x10^{9} \pm 2.0x10^{8}$

$^{a, b, c, \ldots}$ denote the significant differences ($p < 0.05$) in the same column.
^{NS} denotes no significant differences ($p > 0.05$).

Table 6. Quantitative evaluation of the taste of yoghurt in the aspects of overall preference and degree of sourness.

Strain / binder	Preference	Sourness
Yoghurt starter	$6.95^{b} \pm 0.17$	$4.10^{b} \pm 0.65$
L2-1	$6.25^{c} \pm 0.64$	$4.25^{a} \pm 0.49$
Yoghurt starter + pectin	$5.10^{c} \pm 0.62$	$3.80^{b} \pm 0.48$
L2-1+ pectin	$7.26^{a} \pm 0.19$	$3.95^{b} \pm 0.46$
Yomost®	$7.30^{a} \pm 0.72$	$4.50^{a} \pm 0.47$

$^{a, b, c, \ldots}$ denote the significant differences ($p < 0.05$) in the same column.

73-1 could not produce good quality yoghurt, since the curd was not firmly formed and the whey seperated. The optimal concentration of sugar in the production was 10%, since this yielded the required amount of lactic acid and a suitable taste. The optimal time of fermentation was 24 h. Fermentation for 48 h was too long, and the yoghurt produced was too sour. With 10% sugar concentration and incubation at 37°C for 24 h, the quality of yoghurt produced by L2-1 was better than that of 73-1. The pHs of products were 3.62, 3.75, and 3.70; and lactic acid concentrations were 0.82, 0.60 and 0.92 mg/ml while the viscosities were 1203.11, 675.07, and 1344.51 mps (Table 4). The results of products fermented for 48 h are shown in Table 5. Statistical analysis indicated that the pH, lactic acid concentration and viscosity were affected by the content of sugar and the type of starter cultures ($p \leq 0.05$). Yoghurt from strain 73-1 was lumpy and the curd and whey seperated. For the preparation of drinking yoghurt , product of L2-1 and mixed powder (pectin) yielded good quality drinking yoghurt. The levels of preference for the product L2-1 with pectin were similar to Yomost® (a commercial yoghurt product) as shown in Table 6.

Strains L2-1 and 73-1 were found to be L-(+) lactic acid producing strains that could be used as starter for fermented vegetables. Only L2-1 was a good starter for fermented yoghurt. These bacteria showed antibacterial activity against *Staphylococcus*

aureus. Their fermented products contained L(+)-lactic acid which could be metabolized in human body (2). The starter cultures in this study are useful for the production of palatable fermented vegetables and yoghurt. From this study, we have found an optimal method and condition for the homemade production of pickled vegetables and yoghurt.

References

1. B. Fred and F. H. Henery. Using lactic acid bacteria to improve the safety of minimally processed fruits and vegetables. *Food Technol.*, 51: 44-48 (1997).
2. Y. Nakazawa and A. Hosono. *Function of Fermented Milk: Challenges for the Health Sciences.* Translated by B. W. Howells. Elsevier Science Publishers, 1992.
3. A. Rolf, C. Erikson, B. Ann, and O. Theander. Lactic acid fermentation of fresh and stored carrot: Chemical, microbiological and sensory evaluation of products. *Lebensm Wiss U-Technol.* 23: 34-40 (1990).
4. C. S. Pederson. *Microbiology of Food Fermentations*, 2nd ed., C.T. AVI Publishing, West Port, 1979.
5. M. Daeschel, H. Fleming, and R. Mefecters. Mixed culture fermentation of cucumber juice with *Lactobacillus plantarum* and yeasts. *J. Food. Sci.* 53: 862-872 (1988).
6. W. P. Hammes. Bacterial starter cultures in food production. *Food Biotechnol.* 4: 388-397 (1990).
7. A. Tamine and H. Deeth. Yoghurt technology and biochemistry. *J. Food Prod.* 43: 939 (1980).
8. S. Tanasupawat, T. Ezaki, K. Suzuki, S. Okada, K. Komagata, and M. Kozaki. Characterization and identification of *Lactobacillus pentosus* and *Lactobacillus plantarum* strains from fermented foods in Thailand. *J. Gen. Appl. Microbiol.* 37: 479-494 (1992).
9. P. Atthasampunna. *List of Cultures, TISTR Culture Collection,* 5th ed., Thailand Institute of Scientific and Technological Research, Bangkok MIRCEN, Bangkok, 1995.
10. S. Tanasupawat, S. Okada, and K. Komagata. Lactic acid bacteria found in fermented fish in Thailand. *J. Gen. Appl. Microbiol.* 44: 193-200 (1998).
11. J. C. De Man, M. Rogosa, and M. E. Sharpe. A medium for cultivation of lactobacilli. *J. Appl. Bacteriol.* 23: 13 -135 (1960).
12. S. Tanasupawat and W. Daengsubha. *Pediococcus* species and related bacteria found in fermented foods and related material in Thailand. *J. Gen. Appl. Microbiol.* 29: 487-506 (1983).
13. V. Phalip, P. Schmitt, and C. Divies. A method for screening diacetyl and acetoin producing bacteria on agar plates. *J. Basic Microbiol.* 34: 277-280 (1994).
14. K. Komagata and K. Suzuki. Lipid and cell-wall analysis in bacterial systematics. In R. R. Colwell and R. Grigorava (eds.), *Methods in Microbiology,* Vol. 19, Academic Press, London, 1987, pp. 161-207.
15. S. Okada, T. Toyoda, and M. Kozaki. An easy method for the determination of the optical types of lactic acid produced by lactic acid bacteria. *Agric. Biol. Chem.* 42: 1781-1783 (19).
16. J. Samelis and J. Metaxopoulous. Sakacin B, a bacteriocin produced *Lactobacillus sake* isolated from Greek dry fermented sausages. *J. Appl. Bacteriol.* 76: 475-486 (1994).
17. H. G. Meade and N. P. Chen. *Cane Sugar Handbook,* 10th ed., John Wiley and Sons, Toronto, 1977.
18. R. DeVor, T.-H. Chang, and W. Sutherland. *Statistical Quality Design and Control: Contemporary Concepts and Methods.* Macmillan Press, New York, 1992.
19. M. Mandigan, J. Martinko, and J. Parker. *Brock Biology of Microorganisms*, 8th ed., 2000, pp. 519-524.
20. S. Herbert and S. Joel. *Sensory Evaluation,* 2nd ed., Academic Press, San Diego, 1993.