CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Growth profile, pH changes, sugar utilisation and SCFA production by probiotic *Lb. pentosus* strains

A variety of probiotic bacterial strains have ability to use different kinds of various sugars for growth. Evidence suggests that some prebiotic compounds are capable of promoting the growth of probiotics in the colon, since they can pass the upper intestinal region without being hydrolysed (Kneifel et al., 2000). This study investigated on the growth of probiotic *Lb. pentosus* in modified-MRS media, supplemented various sugars for compared with positive control glucose as carbon source and FOS with validated prebiotic activity. Moreover, sugar utilisation and SCFAs production were investgated.

4.1.1 Growth profiles and pH changes

he growth profiles and pH changes of cultured by *Lb. pentosus* namely DM068, JM0812, JM085, UM054, UM055, VM095, VM096, and YM122 were observed during the incubation period in modified-MRS media. The cultures were incubated under anaerobic conditions at 37 ºC and 24 h for all samples. After incubation, the growth intensities of the strains were examined based on optical density (OD) measurements using a spectrophotometer microplate reader (SPECTROstar Nano) at 600 nm (see Appendix C). The results of these analyses are illustrated in Figure 4.1-4.5 as the means ± SD of the triplicate OD and pH measurements for all strains.

The OD of glucose-MRS medium cultured by 8 *Lb. pentosus* strains was presented in Figure 4.1A. The growth patterns of all strains were not significant different ($p > 0.05$) throughout 24 h. The results showed that all strains were quite similar growth curves. The slopes of OD versus incubation time for all of test strains appeared to be similar. The lag phase shortly lasted for 3 h followed by the logarithmic phase of growth from 3-9 h, and the maximum growth was achieved between 9 and 16 h by *Lb. pentosus* UM054, DM068, and UM055 strains at OD 2.62 ± 0.05, 2.59 ± 0.05, and 2.59 ± 0.04 respectively. It appeared that *Lb. pentosus* strains entered the stationary

phase after 16 h until the end of incubation (24 h). Finally, the OD at 24 h found in range between 2.48 ± 0.03 to 2.53 ± 0.00.

In addition, pH values were shown in Figure 4.1B. Similar to the case of the growth curves, the pH profiles of all strains were not significant different ($p > 0.05$). Changes of pH are related to baterial growth behavior, the pH decline at the fastest rate from 6.46 ± 0.00 to 4.45 ± 0.03 from 3 to 12 h and gradually declined to 3.76 ± 0.01 at the end of incubation. This similarity indicated that glucose has no effect on growth behavior of the tested strains. The bacteria can utilise glucose easily as it is monosaccharide. The decrease in pH over time results from the breakdown of glucose to form lactic acid.

Slizewska and Libudzisz (2001 cited in Goderska et al., 2008) demonstrated that glucose is the best source of carbon for *Lb. acidophilus* strain and Goderska K. et al (2008) proved that among the examined carbon sources, *Lb. acidophilus* strain utilized glucose and saccharose best.

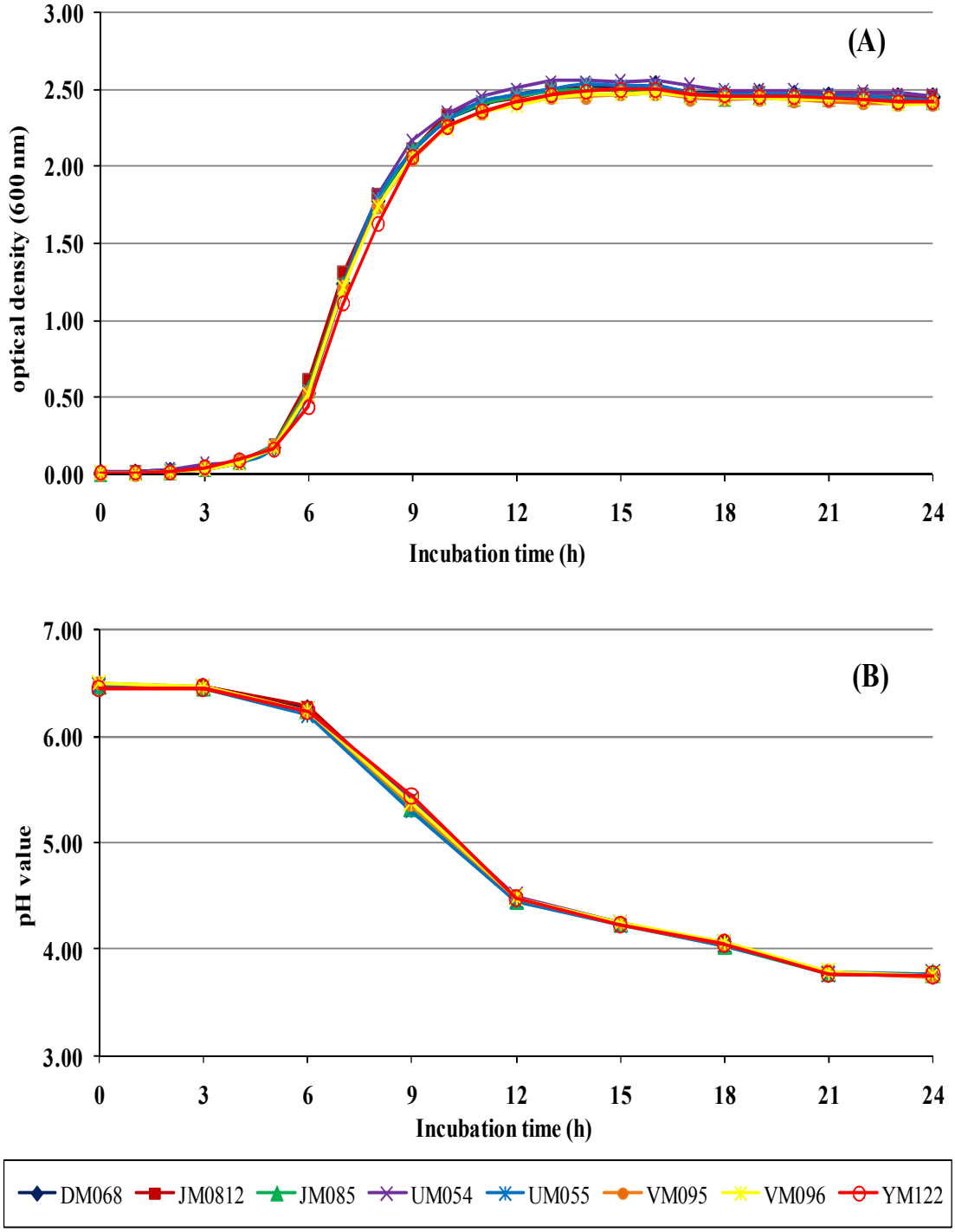

Figure 4.1 Growth profiles (A) and the pH changes (B) in glucose-MRS medium by 8 *Lb. pentosus* strains (◆DM068, ■JM0812, ▲JM085,×UM054,∗UM055, ●VM095, ∗VM096, ○YM122). The results showed mean measurements from triplicate experiments (n = 3). Incubation at 37 ^{o}C for 24 h was performed.

The OD of lactose-MRS media cultured by 8 *Lb. pentosus* strains was showed in Figure 4.2A. We observed that 6 of 8 strains (JM0812, UM054, UM055, VM095, VM096 and YM122) were quite similar growth curves, increased growing significant different ($p < 0.05$). Growth curves of 6 strains above tended to be higher than *Lb. pentosus* DM068 and JM085 strains from 7 - 24 h incubation. It appeared that the lag phase shortly lasted for 3 h followed by logarithmic phase showed a sharp increase between 3-9 h by 6 strains, whereas *Lb. pentosus* DM068 and JM085 strains showed a slightly went up by extend the logarithmic phase and delaying its entrance into the stationary phase. The maximum growth was achieved at 13 h by *Lb. pentosus* VM096, YM122, and JM0182 strains at OD 2.83 ± 0.08, 2.83 ± 0.04, and 2.80 ± 0.07 respectively. It appeared that *Lb. pentosus* strains entered the stationary phase after 16 h until the end of incubation (24 h). Finally, the OD at 24 h was found in range between 1.86 ± 0.16 to 2.57 ± 0.02.

In a similar way, the decrease of pH values (Figure 4.2B) was related to growth curve. The changes pH was significant different ($p < 0.05$) amomg strains from 6 to 24 h incubation. At 24 h incubation, the maximium pH values was achieved at between 3.61±0.01 to 3.63±0.01 by *Lb. pentosus* VM095, VM096, YM122, UM055, and UM054 respectively whereas a slightly changes pH was found at 4.93±0.01 by *Lb. pentosus* DM068 and JM085.

The results indicated that lactose-MRS medium had the differential effect on the growth behavior of the strains by achieved the maximium OD higher compared to glucose-MRS medium. Lactose is a disaccharide or a milk oligosaccharide, a type of nature sugar found in milk and hydrolysed by LAB into galactose and glucose (Venema, 2012) and helps to growth promoting of probiotic bacteria. The decrease in pH over time results from the increasing breakdown of lactose to accumulate lactic acid (Olson and Aryana, 2012). In addition, the experiment results showed that 6 of 8 strains can growth well and has the lower pH values by utilize lactose.

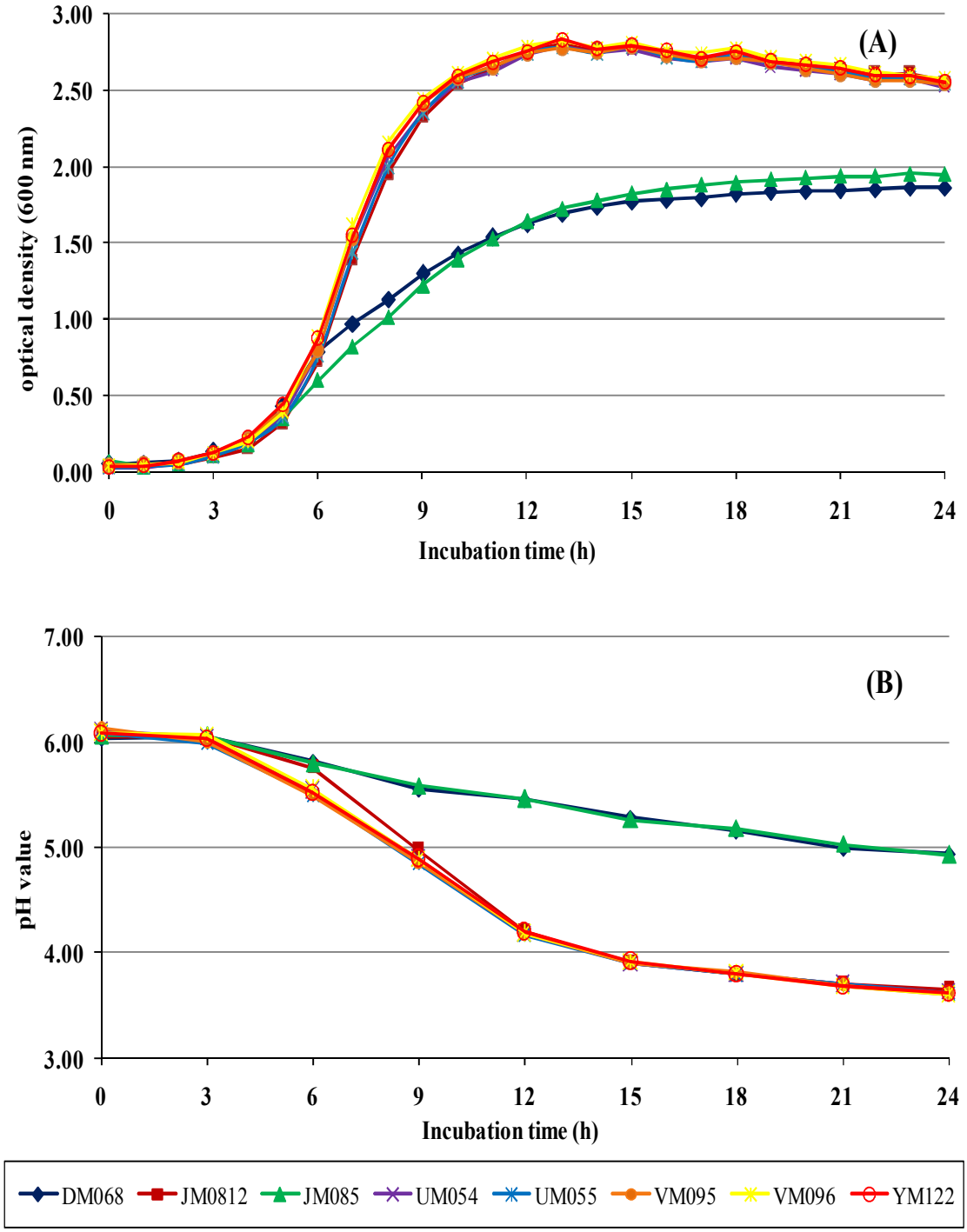

Figure 4.2 Growth profiles (A) and the pH changed (B) in lactose-MRS medium by 8 *Lb. pentosus* strains (◆DM068, ■JM0812, ▲JM085, ×UM054,✳UM055, ●VM095, ✳VM096, ○YM122). The results showed mean measurements from triplicate experiments (n = 3). Incubation at 37 ^{o}C for 24 h was performed.

As shown in Figure 4.3A, eight LAB *Lb. pentosus* strains were compared for their growth in raffinose-MRS medium. The OD was significant different ($p < 0.05$) among strains during 24 h incubation. It was found that 5 strains (YM122, VM096, UM055, UM054 and VM095) showed higher growth rate in raffinose-MRS medium than 3 strains (DM068, JM0812 and JM085) whereas those of 3 strains grown quite well in glucose-MRS medium. It seen that the lag phase shortly lasted for 3 h followed by logarithmic phase showed a sharp increase between 3 h to9 h by 5 strains, then entered the stationary phase after 15 h until 24 h incubation. The maximum growth was achieved between 9 h and 13 h by *Lb. pentosus* YM122, VM096, and UM055 strains at OD 2.93 ± 0.04, 2.93 ± 0.05, and 2.86 ± 0.11 respectively followed by VM095 and UM054. It appeared that *Lb. pentosus* strains entered the stationary phase after 16 h until the end of incubation (24 h). Finally, the OD at 24 h was found in range between 1.99 ± 0.03 to 2.58 ± 0.01. After 13 h, the curve showed decrease in maximum OD levels, indicating that raffinose may be more rapidly exhausted.

Similar the growth pattern, the pH values were significant different ($p < 0.05$) among the tested strains during 24 h incubation (Figure 4.3B). The pH rapidly declined at the 3 to 12 h from 6.20 ± 0.01 to 4.25 ± 0.01 due to LAB substrate consumption for growing and producing of lactic acid at the same time with high growth rate. At the end of incubation 24 h, pH value arranged by those 5 strains as above was approximately 3.70.

Above results demonstrated that raffinose-MRS medium had effect to promote on the growth behavior of some strains in this study by achieved the maximium OD higher compared to glucose-MRS medium. The YM122, VM096, UM055, VM095 and UM054 can growth in raffinose-MRS medium better than DM068, JM0812 and JM085 strains. Raffinose is a oligosaccharide in soybean, a trisaccharide composed of galactose, glucose, and fructose. However, it is the one of main oligosaccharide in soybean that is not digestable by human body but can be hydrolyzed by the enzyme alpha-galactosidase from LAB (Wang et al, 2003).

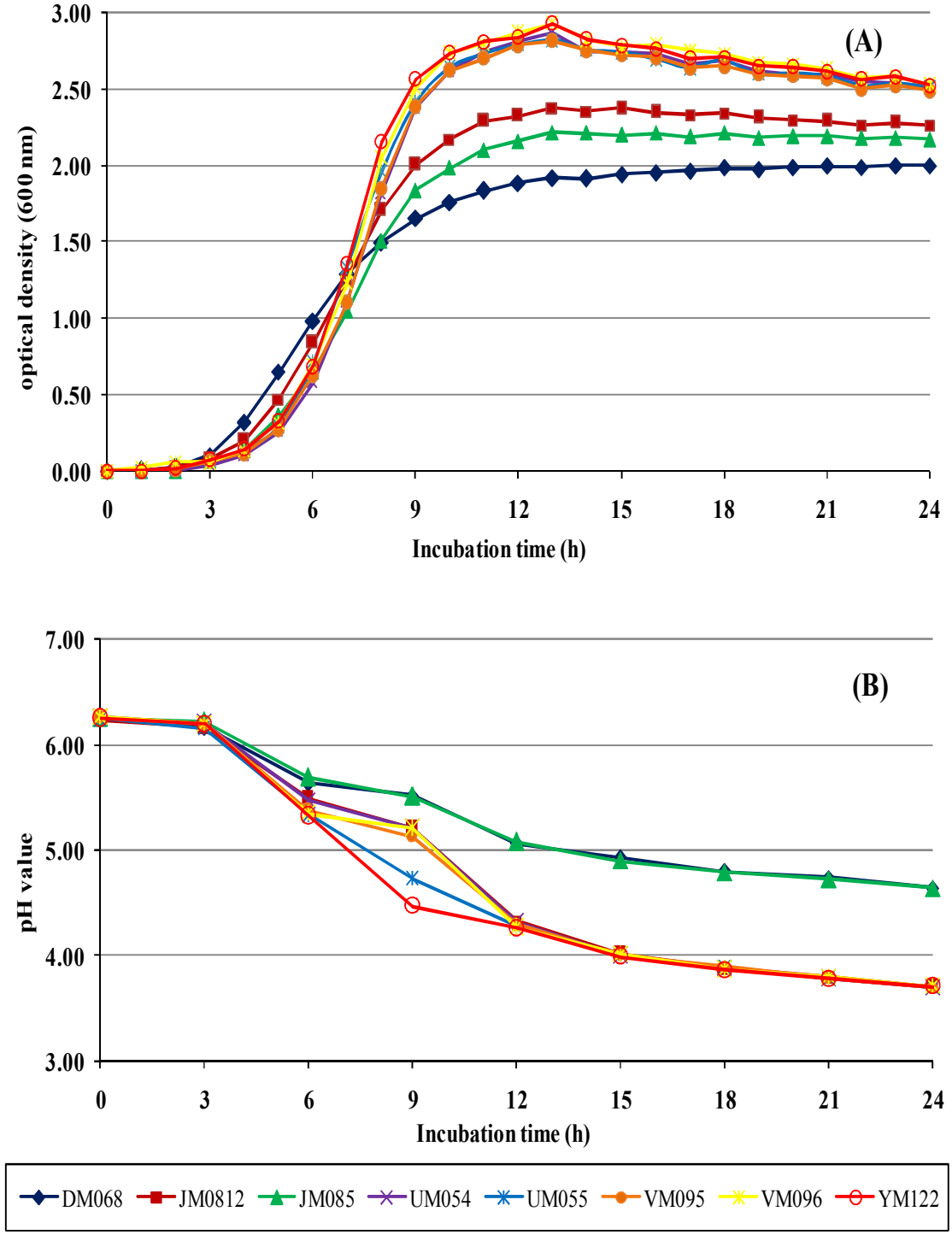

Figure 4.3 Growth profiles (A) and the pH changed (B) in raffinose-MRS medium by *Lb. pentosus* 8 strains (◆DM068, ■JM0812, ▲JM085, ×UM054,✳UM055, ●VM095, ✳VM096, ○YM122). The results showed mean measurements from triplicate experiments (n = 3). Incubation at 37 ^{o}C for 24 h was performed.

As shown in Figure 4.4A-B, the data of growth profiles and changes pH of *Lb. pentosus* 8 strains were observed in FOS-MRS medium. It was found that 6 of 8 strains used in this study including *Lb. pentosus* JM0182, UM054, UM055, VM095, VM096 and YM122 grew very well whereas the strains DM068 and JM085 were slightly growing in FOS-MRS medium. Six strains above increased growing significant different ($p < 0.05$) compared with DM068 and JM085 after 6 h of incubation period. The lag phase is shortly lasted for 3 h and the logarithmic phase showed a sharp increase between 3 to 9 h incubation. The exponential phase contained after 9 h. The maximum cells density was achieved at 13 h by *Lb. pentosus* UM054, JM0812, and UM055 strains at OD 2.95 ± 0.03, 2.94 ± 0.02, and 2.93 ± 0.02 respectively followed by VM096 and VM095. At the end 24 h, the OD was found in range between 1.98 to 2.61.

Similar the growth pattern, the pH values were significant different ($p<0.05$) among the test strains at 6 h until end of incubation at 24 h. The pH rapidly declined at the 3 to 12 h from 6.22 ± 0.05 to 4.20 ± 0.02 by 6 strains as above. At the end of incubation 24 h, the pH declined to 3.66 ± 0.01. FOS in MRS media has the effect on the growth behavior of the some test strains in this study. The JM0812, UM054, VM096, UM055, VM095, YM122 can grow in FOS-MRS medium better than DM068 and JM085 strains.

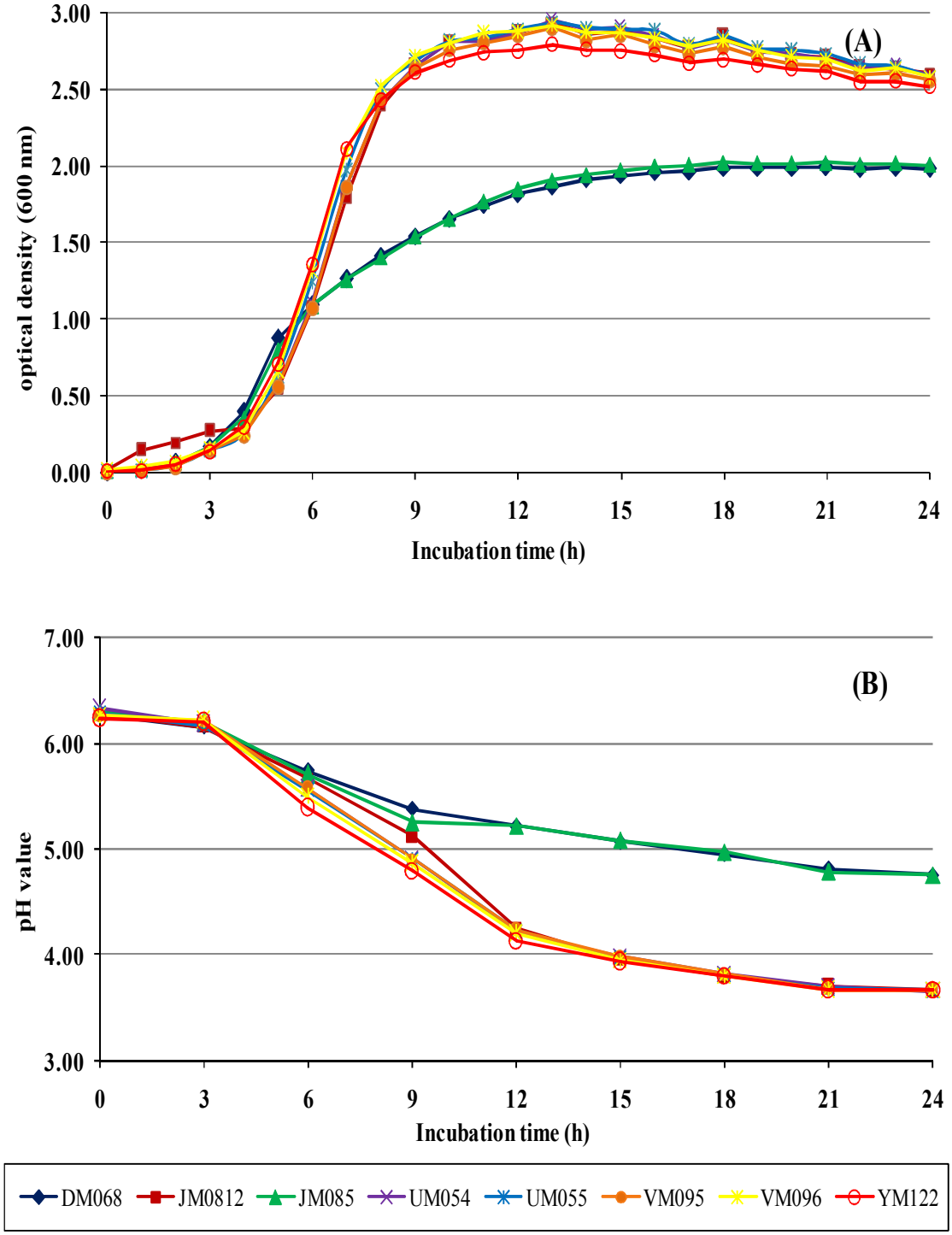

Figure 4.4 Growth profiles (A) and the pH changed (B) in FOS-MRS medium by *Lb. pentosus* 8 strains (◆DM068, ■JM0812, ▲JM085, ×UM054,✳UM055, ●VM095, ✳VM096, ○YM122). The results showed mean measurements from triplicate experiments (n = 3). Incubation at 37 ^{o}C for 24 h was performed.

The growth curves obtained for 8 *Lb. pentosus* strain in different sugars; lactose, raffinose were summaried in Figure 4.5. The experimental medium was compared with glucose as a control in the MRS media and FOS with validated prebiotic activity and no negative control-MRS broth without any carbon source in this study. The results found that the maximum growth differed between sugars tested as a direct carbon source.

The growth rates of *Lb. pentosus* DM068 and JM085 were achieved maximum OD in glucose higher than raffinose, FOS, and lactose significant different ($p < 0.05$). However, the pH values of glucose-MRS declined faster than those of sugars. Glucose was found to be the best carbon source for DM068 and JM085 strains. The maximum growth found at 24 h incubation. Glucose, raffinose, FOS, and lactose reached OD levels of 2.52, 2.00, 1.98, and 1.86, respectivly by DM068 (significant different) whereas, pH values was achieved 3.76, 4.64, 4.76, and 4.93 respectively. Similary, in case of the JM085 strain, the maximum growth found at 15 h, reached OD levels of 2.56, 2.20, 1.97, and 1.82, respectively for gluclose, raffinose, FOS, and lactose-MRS medium. The pH values were 4.24, 4.90, 5.08, and 5.26 respectively. The ending OD and pH values ranges between 1.95-2.49 and 4.93- 3.77.

In better case of JM0812 culture, FOS and lactose were found to be the best carbon source than glucose, whereas raffinose promoted growth lower than glucose by this strain. The maximum growth at 15 h for FOS, lactose, glucose and raffinose-MRS reached OD levels of 2.88, 2.77, 2.57, and 2.37 (significant different) whereas, pH value was achieved 3.95-4.25. On the other hand, the UM054, UM055, VM095, VM096 and YM122 strains had ability to growth in lactose, raffinose and FOS-MRS medium better than glucose. The growth patterns of all 5 strains were quite similarly. Lactose, raffinose and FOS enhanced the growth intensities of 5 strains whereas DM068 and JM085 were not growing well. The maximum growth at 12-15 h found in oligosaccharide sugars, reached approximately 2.72-2.91 whereas OD in glucose reached 2.54-2.62 (significant different). In addition, pH values at 24 h were achived 3.60-3.70.

However, Sánzhes-Zapata et al. (2013) was reported that a very limited growth was observed in the negative control medium (MRS without any carbon source). Glucose was found to be the best substrate to *Lb. acidophilus* grows, followed by FOS and Tiger nut milk (TNLC). Su et al. (2007) reported that glucose was better than FOS in supporting the growth of *Lb. acidophilus*L10. Main sugars in TNLC include sucrose,

fructose, and glucose (in similar amounts) and small amounts of raffinose and glycerol (Sánzhes-Zapata et al., 2013). On the other hand, Crittenden et al. (2001) demonstrated that *Bifidobacterium* grew better with FOS, galactooligosaccharides and xylooligosaccharides than on monosaccharides (as glucose). However, our results showed lactose, raffinose, and FOS-MRS medium enhanced the growth intensities most of tested strains than glucose during the exponential phase of growth. As a result, the effectiveness of a prebiotic depends, therefore on its ability to be selectively fermented by and to support growth of specific targeted organisms (Huebner et al., 2007).

Sugars such as oligosaccharide can be used as a carbon source to promote the growth of *Lb. pentosus* strains. Most of tested strains can be growing in oligosaccharide, they exhibit a faster growth than when grown in monosaccharide (as glucose). These results are also supported by the lower pH and by the faster organic acid production, confirming the relevant property of oligosaccharide as a carbon source for probiotic bacteria growth. These results indicate that soya milk can have a potential to be used for probiotic soya beverages as a fermentable product, but more studies are required to establish the doses, stability and its compatibility with other probiotic microorganisms.

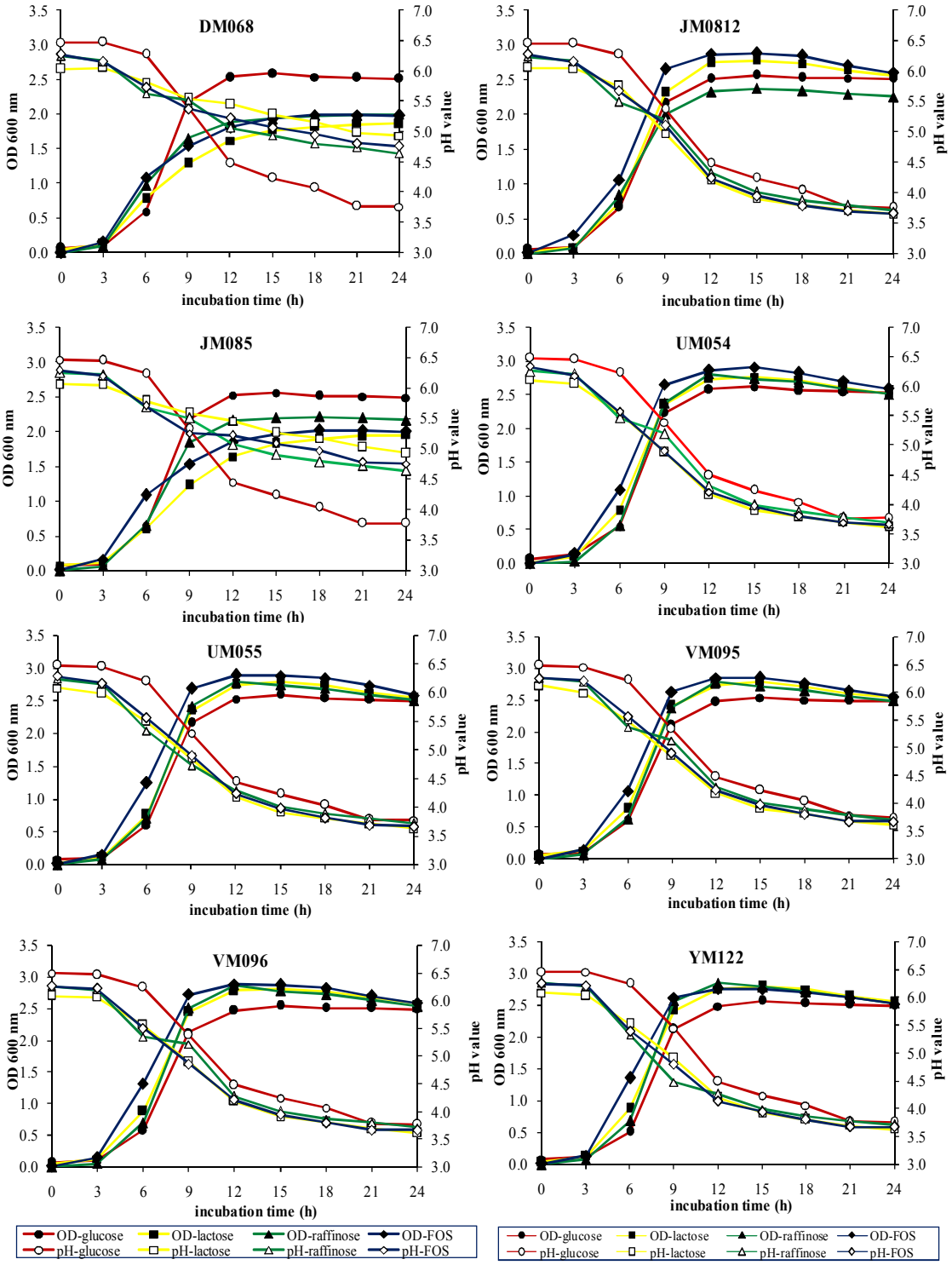

Figure 4.5 Growth behaviors of 8 *Lb. pentosus* strains in MRS with various sugars, lactose (OD= ■; pH=□), raffinose (OD=▲; pH=△), and FOS (OD=◆; pH=◇) as a carbon source and gluclose-MRS medium (OD=●; pH=○) as control. Results were shown as mean measurements from triplicate experiments (n = 3).

4.1.2 Sugar utilisation and SCFAs production by probiotic *Lb. pentosus* strains

4.1.2.1 Glucose utilisation

The capacities of glucose utilisation, lactic acid production and SCFAs production by 8 *Lb. pentosus* strains, namely DM068, JM0812, JM085, UM054, UM055, VM095, VM096, and YM122 in MRS media containing 2% glucose are summarized in Table 4.1. Glucose decreased over 24 h whereas lactic acid increased. The capacity of glucose utilisation for all test strains have quite similar profile with the greater consumption more than 90% (decrease from 17.61 ± 1.66 to 0.91 ± 0.01 mg/mL) of initial concentration of glucose (17.61-16.69 mg/mL in MRS medium). In case of lactic acid production, all strains could produce high amount of lactic acid after first 6 h incubation. The concentration of lactic acid was highest in *Lb. pentosus* DM068 (17.54 ± 0.16 mg/mL) followed by VM095, YM122, and UM054 17.19 ± 0.13, 17.05 ± 0.08 and 17.00 ± 0.05 mg/mL, respectively in MRS media at 24 h incubation.

The ways of probiotic properties are still a hightlight for probiotic selection. Some probiotics influence the change in the profile of fatty acids. SCFAs are the main end-products, which produce by probiotic fermentation from carbohydrate. The major SCFAs are acetic, propionic, butyric and valaric acid. The principal substrates include a wide variety of dietary residues, the main ones being prebiotic sugars.

SCFAs production (acetic, propionic, butyric, iso-butyric, and n-valeric acid) were significant different ($p < 0.05$) among strains at 24 h incubation. Acetic acid produced was ranged between 79.09 ± 1.64 to 90.08 ± 0.44 µmol/mL with YM122 strain showed highest acetic acid produced. Never the less, the production of other SCFAs in the end of incubation with high amount of propionic acid was produced by VM096 (38.31 ± 0.23 µmol/mL), butyric acid by YM122 (37.82 ± 0.12 µmol/mL), iso-butyric by DM068 and JM085 (16.33 ± 0.07 and 16.32 ± 0.02 µmol/mL, respectively), and n-valeric acid by UM054 (181.77 ± 0.00 µmol/mL).

Table 4.1 The capacity of glucose utilisation, lactic acid, and SCFAs production by probiotic *Lb. pentosus* 8 strains in glucose-MRS medium 24 h incubation period.

Incubation Time (h)	*Lb. pentosus* Strains	The capacity of glucose utilisation, lactic acid, and SCFAs production by probiotic *Lb. pentosus* 8 strains						
		Sugar contents (mg/mL)	Lactic acid (mg/mL)	SCFAs (µmol/mL)				
		D(+)Glucose	Lactic acid	Acetic acid	Propionic acid	Butyric acid	Iso-butyric acid	n-valeric acid
0	DM068	17.18±0.05ns	0.17±0.05ns	0.08±0.01b	99.75±10.97b	5.60±0.11d	nd	1500.28±74.04a
	JM0812	17.31±0.38ns	0.14±0.00ns	0.08±0.00b	73.90±5.11cd	2.61±0.01f	nd	543.41±20.51d
	JM085	17.61±1.66ns	0.14±0.01ns	78.20±13.10b	96.22±0.37b	9.93±0.56b	nd	880.95±32.23c
	UM054	17.32±0.26ns	0.23±0.11ns	104.20±9.13a	152.30±15.17a	nd	nd	1041.90±138.54bc
	UM055	17.34±0.19ns	0.14±0.00ns	82.82±1.02b	90.90±10.37bc	10.55±0.02a	nd	1115.59±31.22b
	VM095	17.26±0.19ns	0.14±0.00ns	78.64±4.61b	50.40±5.67f	4.88±0.20e	nd	988.08±37.86bc
	VM096	16.91±0.19ns	0.14±0.00ns	78.47±0.21b	60.33±2.78df	6.05±0.45cd	nd	583.01±77.45d
	YM122	16.69±0.29ns	0.20±0.00ns	81.78±0.73b	65.81±4.64df	6.60±0.09c	nd	1036.66±0.53bc
6	DM068	17.04±0.98ns	0.50±0.10d	97.90±8.42ns	82.36±6.49a	9.72±0.71ns	nd	943.29±52.63bc
	JM0812	16.98±1.04ns	0.92±0.06a	100.86±6.12ns	76.30±1.02a	9.33±0.00ns	nd	830.65±78.40c
	JM085	17.61±0.48ns	0.61±0.04bcd	100.71±0.25ns	82.45±2.83a	8.59±0.05ns	nd	1036.89±49.65ab
	UM054	17.11±0.12ns	0.67±0.09bc	100.55±1.52ns	78.32±1.04a	8.97±0.11ns	nd	1104.11±89.68a
	UM055	16.93±0.12ns	0.74±0.01b	95.97±1.74ns	77.41±2.50a	9.69±0.30ns	nd	1013.32±43.67ab
	VM095	17.06±0.00ns	0.70±0.00bc	100.46±0.43ns	78.80±1.43a	10.19±0.09ns	nd	984.85±4.41ab
	VM096	17.16±0.15ns	0.57±0.04cd	98.75±1.80ns	76.46±2.26a	10.26±0.06ns	nd	984.61±8.25ab
	YM122	17.14±0.14ns	0.57±0.07cd	95.48±0.45ns	56.25±4.45b	8.16±2.73ns	nd	569.62±24.28d
12	DM068	9.36±0.01ab	8.51±0.12bc	102.13±3.42ab	31.92±2.35e	15.12±0.00b	nd	122.97±30.01d
	JM0812	9.39±0.08ab	8.18±0.00c	103.08±1.25a	50.13±3.24c	14.61±0.00b	nd	413.77±51.74c
	JM085	9.18±0.02ab	8.78±0.28ab	96.02±1.78ab	42.72±1.53cd	11.64±2.91b	nd	163.69±20.46d
	UM054	9.53±0.24a	8.38±0.21bc	99.37±6.19ab	43.69±5.32cd	27.84±3.25a	nd	482.08±0.91bc
	UM055	9.09±0.39b	8.96±0.08a	101.13±4.18ab	39.61±0.78d	4.09±0.45c	nd	503.86±67.40b
	VM095	9.45±0.01ab	8.50±0.27bc	103.13±3.59a	37.80±0.67de	7.13±0.67c	nd	687.63±18.11a
	VM096	9.24±0.03ab	8.51±0.16bc	94.66±1.68b	62.95±4.31b	7.69±0.62c	nd	623.94±23.04a
	YM122	9.53±0.09a	8.57±0.04abc	96.03±0.47ab	96.25±3.32a	13.91±0.45b	nd	nd

Table 4.1 Continued

Incubation Time (h)	*Lb. pentosus* Strains	The capacity of glucose utilisation, lactic acid, and SCFAs production by probiotic *Lb. pentosus* 8 strains						
		Sugar contents (mg/mL)	Lactic acid (mg/mL)	SCFAs (µmol/mL)				
		D(+)Glucose	Lactic acid	Acetic acid	Propionic acid	Butyric acid	Iso-butyric acid	n-valeric acid
24	DM068	0.94±0.01a	17.54±0.16a	79.82±2.23c	25.18±5.14c	5.95±0.00dc	16.33±0.07a	110.02±7.98c
	JM0812	0.92±0.00b	16.92±0.08c	83.82±1.99bc	34.43±2.29ab	5.63±0.12dc	15.82±0.02b	122.13±8.71b
	JM085	0.91±0.01b	16.97±0.04c	80.71±1.52bc	34.86±1.04ab	nd	16.32±0.02a	124.64±0.97b
	UM054	0.93±0.00b	17.00±0.05bc	84.81±3.69b	33.15±1.97ab	nd	15.42±0.03c	181.77±0.00a
	UM055	0.91±0.00b	16.90±0.03c	79.09±1.64c	32.69±0.77ab	nd	15.87±0.02b	128.98±0.00b
	VM095	0.92±0.01b	17.19±0.13b	81.88±0.54bc	36.30±4.04ab	8.70±0.15b	14.80±0.08d	nd
	VM096	0.91±0.01b	16.99±0.04bc	82.36±0.91bc	38.31±0.23a	7.98±0.62b	13.92±0.03e	34.96±0.00d
	YM122	0.92±0.01b	17.05±0.08bc	90.08±0.44a	29.72±4.89bc	37.82±0.12a	nd	43.75±0.77d

Note: the mean values ± SD in the same column with the same time of each strain follow with different small letters were significant different ($p < 0.05$), ns= non significant, nd= non detectable

4.1.2.2 Lactose utilisation

The capacity of lactose utilisation by *Lb. pentosus* strains DM068, JM0812, JM085, UM054, UM055, VM095, VM096, and YM122 in MRS medium contains 2% lactose are presented in Table 4.2. The utilisation of lactose in MRS medium was significant different ($p < 0.05$) among strains. Never the less, lactose decreased over time, the small amount of D (+) glucose (range 0.75 ± 0.02 to 1.26 ± 0.04 mg/mL) and D (+) galactose (range 0.07 ± 0.01 to 0.70 ± 0.05 mg/mL) were found in MRS medium due to breakdown of molecule lactose formed to glucose-galactose by bacteria. In the same time, lactic acid and SCFAs was produced from lactose consumption by *Lb. pentosus* strains. During 24 h incubation, the concentration of lactose decreased from 34.50 ± 0.00 to 11.20 ± 0.06 mg/mL and from 36.54 ± 1.59 to 11.57 ± 0.49 mg/mL with highest capacity of lactose utilisation by YM122 and VM096, respectively. However, DM068 and JM085 strains had lower capacity of lactose utilisation.

The lactic acid and SCFAs production by 8 *Lb. pentosus* strains in MRS media contains 2% lactose are also summarized in Table 4.2 In case of lactic acid production, 6 of 8 strains could produced high amount of lactic acid after 6 h incubation except DM068 and JM085 strains. There were significant different ($p < 0.05$) highest lactic acid produced between range 17.46 ± 0.84 to 18.13±0.13 mg/mL at 24 h incubation. Acetic acid decreased during time, the concentration of acetic acid in JM085 and DM068 were high amount 99.33 ± 5.16 and 92.18 ± 5.81 µmol/mL, respectively 24 h. For other SCFAs production, the concentration of propionic, butyric, iso-butyric, and n-valeric acid were significant different ($p < 0.05$) among strains at 24 h incubation. The high concentration of propionic acid that 69.47 ± 1.52, 66.24 ± 5.27 and 64.70 ± 3.38 µmol/mL by JM085, DM068 and VM095, respectively. The high amount of butyric acid was produced by JM085 (16.35 ± 0.49 µmol/mL), iso-butyric produced by only 3 strains that JM085, DM068 and JM0812 (11.70 ± 1.00 to 24.18 ± 1.63 µmol/mL), and n-valeric acid by JM085, JM0812, and VM095 (299.08 ± 7.19 to 338.53 ± 27.84 µmol/mL).

Table 4.2 The capacity of lactose utilisation, lactic acid, and SCFASs production by probiotic *Lb. pentosus* 8 strains in Lactose-MRS medium 24 h incubation period

Incubation Time (h)	*Lb. pentosus* Strains	The capacity of lactose utilisation, lactic acid, and SCFAs production by probiotic *Lb. pentosus* 8 strains								
		Sugar contents (mg/mL)			Lactic acid (mg/mL)	SCFAs (µmol/mL)				
		lactose	D(+)glucose	galactose	Lactic acid	Acetic acid	Propionic acid	Butyric acid	Iso-butyric acid	n-valeric acid
0	DM068	25.67±1.75b	0.59±0.01d	0.58±0.03c	0.32±0.10b	99.20±4.11bc	70.85±0.88c	42.12±8.42bc	nd	794.89±75.46bc
	JM0812	28.32±2.57b	0.74±0.12bcd	0.94±0.10b	0.65±0.29ab	115.37±3.40a	76.81±5.84bc	11.38±0.44ef	nd	709.26±26.39c
	JM085	28.90±0.52b	0.85±0.06abc	0.99±0.05b	0.42±0.04ab	100.37±1.92b	85.06±3.27b	16.15±7.82de	nd	711.84±50.90c
	UM054	26.85±0.15b	0.72±0.08bcd	0.90±0.04b	0.68±0.21a	92.43±0.21c	99.70±0.05a	nd	nd	1117.29±7.22a
	UM055	27.75±1.64b	0.65±0.21cd	0.91±0.09b	0.44±0.04ab	84.09±4.97d	89.33±9.98ab	70.64±11.86a	nd	853.52±15.19b
	VM095	28.95±2.11b	0.82±0.03abcd	0.98±0.06b	0.45±0.02ab	74.22±0.17e	70.94±2.93c	47.92±0.90b	nd	1085.59±77.79a
	VM096	36.54±1.59a	0.97±0.01a	1.20±0.07a	0.51±0.06ab	69.40±2.62ef	48.04±0.23d	28.36±1.35cd	nd	711.81±75.10c
	YM122	34.50±0.33a	0.89±0.00ab	1.18±0.02a	0.52±0.01ab	64.17±3.09f	46.99±8.15d	36.93±1.77bc	nd	536.80±13.94d
6	DM068	25.05±1.11ab	1.18±0.05c	0.42±0.07b	3.81±0.25a	69.39±3.16a	54.54±12.32b	12.83±0.31d	nd	68.83±10.59e
	JM0812	26.58±1.32ab	1.17±0.06c	0.54±0.00ab	1.10±0.04b	66.93±1.80ab	68.38±1.49a	32.86±2.66a	nd	187.14±3.97d
	JM085	28.47±1.78a	1.21±0.04abc	0.59±0.04a	1.21±0.02b	32.93±1.88c	9.86±1.44d	7.82±1.04e	nd	40.93±0.00f
	UM054	24.49±0.77ab	1.18±0.04bc	0.43±0.02b	4.14±0.04a	32.97±2.37c	41.82±1.22c	9.78±0.12de	5.80±1.07	nd
	UM055	25.32±0.42ab	1.35±0.09ab	0.50±0.07ab	3.73±0.24a	60.77±0.63b	54.80±1.75b	17.70±2.11c	11.05±2.81	63.86±9.02ef
	VM095	25.53±0.85ab	1.25±0.05abc	0.46±0.03ab	4.20±0.37a	63.56±4.06ab	60.99±2.99ab	24.77±0.37b	24.42±1.30	478.98±22.37b
	VM096	25.66±1.71ab	1.36±0.12a	0.51±0.11ab	3.93±0.31a	62.30±3.43ab	59.92±3.15ab	20.33±2.42bc	15.73±1.07	319.79±18.50c
	YM122	25.74±1.48ab	1.24±0.04abc	0.48±0.00ab	3.91±0.49a	63.50±5.58ab	62.25±4.17ab	22.46±3.24b	6.45±0.17	539.00±0.00a
12	DM068	26.82±2.91a	1.24±0.08a	0.74±0.09a	1.98±0.03b	60.14±4.82a	68.20±9.18a	43.95±0.68a	nd	345.97±62.02a
	JM0812	19.10±0.83b	0.97±0.04b	0.18±0.03b	10.76±0.46a	52.92±3.49ab	68.58±4.35a	7.87±0.09d	nd	nd
	JM085	26.96±1.47a	1.29±0.07a	0.74±0.02a	2.08±0.18b	59.49±1.38a	68.09±0.12a	17.73±2.50b	18.13±0.00	202.37±7.93bc
	UM054	18.21±0.91b	0.91±0.02b	0.13±0.01b	11.09±0.11a	48.09±4.31b	46.73±2.79b	9.46±1.75cd	nd	nd
	UM055	18.78±0.91b	0.98±0.09b	0.18±0.08b	11.29±0.12a	51.67±2.38b	70.73±5.04a	11.38±1.11cd	nd	nd
	VM095	19.29±0.42b	0.95±0.01b	0.16±0.03b	10.85±0.70a	51.35±2.36b	68.01±5.58a	11.13±1.22cd	nd	157.36±0.00c
	VM096	19.74±1.21b	1.01±0.01b	0.20±0.03b	10.46±0.51a	53.21±0.69ab	68.82±1.67a	8.29±0.14d	7.79±0.25	229.08±14.69b
	YM122	19.43±2.01b	0.99±0.12b	0.17±0.06b	10.81±0.13a	53.98±3.17ab	71.67±6.38a	13.33±3.83c	7.62±0.26	332.82±28.19a

Table 4.2 Continued

Incubation Time (h)	*Lb. pentosus* Strains	The capacity of lactose utilisation, lactic acid, and SCFAs production by probiotic *Lb. pentosus* 8 strains								
		Sugar contents (mg/mL)			Lactic acid (mg/mL)	SCFAs (µmol/mL)				
		lactose	D(+)glucose	galactose	Lactic acid	Acetic acid	Propionic acid	Butyric acid	Iso-butyric acid	n-valeric acid
24	DM068	25.45±0.91a	1.26±0.04a	0.70±0.05a	3.61±0.11c	92.18±5.81a	64.70±3.38a	8.29±0.04i	20.55±0.78b	114.18±22.28d
	JM0812	13.25±0.71c	0.75±0.03d	0.06±0.00c	17.51±0.82a	54.11±2.73b	40.02±12.54bc	7.34±0.13j	11.70±0.00c	309.71±26.80ab
	JM085	22.55±0.54b	1.10±0.03b	0.24±0.03b	7.64±0.66b	99.33±5.16a	69.47±1.52a	16.35±0.49a	24.18±1.63a	338.53±27.84a
	UM054	10.86±0.08d	0.84±0.01cd	0.12±0.00c	17.08±0.13a	50.26±0.51b	21.06±0.92d	11.47±0.57e	nd	238.84±7.71c
	UM055	11.74±0.61d	0.84±0.10cd	0.13±0.10bc	17.90±0.53a	54.56±0.67b	38.34±9.19bc	15.13±0.38b	nd	282.25±6.18bc
	VM095	12.29±0.77cd	0.78±0.00cd	0.07±0.00c	18.13±0.13a	55.72±0.84b	66.24±5.27a	10.46±0.00f	nd	299.08±7.19ab
	VM096	11.57±0.49d	0.90±0.09c	0.17±0.06bc	17.75±1.04a	53.67±5.34b	45.78±6.26b	13.33±0.04c	nd	273.88±23.78bc
	YM122	11.20±0.06d	0.75±0.02d	0.07±0.01c	17.46±0.84a	54.23±0.84b	26.28±0.18cd	12.56±0.34d	nd	282.44±0.85bc

Note: the meanvalues±SD in the same column, in the same time of each strain with different small letters were significant different ($p<0.05$) ns= non significant, nd= non detectable

4.1.2.3 Raffinose utilisation

The raffinose utilisation by *Lb. pentosus* strains DM068, JM0812, JM085, UM054, UM055, VM095, VM096, and YM122 in MRS medium contains 2% raffinose are presented in Table 4.3. The utilisation of raffinose in MRS medium was significant different ($p < 0.05$) among strains at the incubation period. The concentration of raffinose decreased during incubation. At the same time, the concentration of monosaccharide galactose and glucose increased in the culture media. Due to molecule raffinose is trisaccharide formed to glucose + galactose + fructose then raffinose could be breakdown by *Lb. pentosus* strains. However, a small amount of glucose (range 1.79 ± 0.07 to 2.68 ± 0.00 mg/mL) was found only at the initial time incubation maybe molecule raffinose breakdown to form monosaccharide from the preparation culture media process whereas fructose did not detect in this experiment. The amount of raffinose decreased significant ($p < 0.05$) among strains. In the same time, lactic acid and SCFAs was produced from raffinose consumption by bacteria strains. During 24 h incubation, the concentration of raffinose decreased with highest capacity of raffinose utilisation by UM055 and YM122 from 28.96 ± 2.52 to 10.49 ± 0.07 mg/mL and from 28.36 ± 3.58 to 10.30 ± 0.00 mg/mL, respectively whereas the amount of galactose changed from 5.40 ± 0.57 to 2.62 ± 0.00 mg/mL. However, DM068 and JM085 strains had lower capacity of lactose utilisation.

Table 4.3 also present the lactic acid and SCFAs production by *Lb. pentosus* 8 strains in MRS medium contains 2% raffinose. In case of lactic acid production, 6 of 8 strains could produce high amount of lactic acid in the first 6 h incubation except DM068 and JM085 strains. There were increased significant different ($p < 0.05$) highest produced amount of lactic acid between range 17.48 ± 0.840 to 17.93 ± 0.23 mg/mL at 24 h incubation. Also, acetic acid increased during time incubation, but at the end 24 h the concentration of acetic acid dropped from 12 h, it found high amount of acetic acid were 98.49 ± 8.37, 93.31 ± 0.18 and 89.02 ± 9.43 μmol/mL by VM096, UM055, and VM095, respectively at 24 h incubation.

For other SCFAs production, the concentration of propionic, butyric, iso-butyric, and n-valeric acid were significant ($p < 0.05$) among strains at 24 h incubation. The high concentration of propionic acid was 29.49 ± 2.79 μmol/mL by JM0812. The high amount of butyric acid was produced by UM054 (19.98 ± 0.54 μmol/mL), iso-

butyric by JM085 (15.55 ± 2.33µmol/mL), and n-valeric acid by JM0812 (624.22 ± 3.74 µmol/mL).

Table 4.3 The capacity of raffinose utilisation, lactic acid, and SCFAs production by probiotic *Lb. pentosus* 8 strains in Raffinose-MRS medium 24 h incubation period.

Incubation Time (h)	*Lb. pentosus* Strains	The capacity of raffinose utilisation, lactic acid, and SCFAs production by probiotic *Lb. pentosus* 8 strains									
		Sugar contents (mg/mL)				Lactic acid (mg/mL)	SCFAs (μmol/mL)				
		raffinose	D(+) glucose	galactose	D(-) fructose	Lactic acid	Acetic acid	Propionic acid	Butyric acid	Iso-butyric acid	n-valeric acid
0	DM068	23.81±0.63bc	1.79±0.07c	3.57±0.82bc	nd	0.43±0.21ab	93.22±2.97b	268.99±44.74a	32.80±3.28b	nd	414.12±46.93c
	JM0812	21.40±0.67cd	nd	5.40±0.57a	nd	0.46±0.09ab	nd	nd	nd	nd	nd
	JM085	26.46±1.36ab	2.68±0.00a	4.97±0.76ab	nd	0.29±0.06b	96.49±2.49b	280.14±14.52a	20.86±0.05c	nd	491.42±8.75b
	UM054	18.02±0.86d	1.79±0.00c	5.08±0.81ab	nd	0.77±0.06a	104.97±0.60a	nd	22.11±2.92c	nd	504.25±42.89b
	UM055	28.96±2.52a	2.18±0.18b	4.25±0.63bc	nd	0.62±0.37ab	108.61±0.19a	nd	39.20±1.57a	nd	627.16±6.22a
	VM095	17.80±1.63d	1.92±0.00c	3.15±0.38c	nd	0.25±0.02b	104.75±0.06a	nd	29.81±0.93b	nd	599.43±50.46a
	VM096	18.53±0.04d	nd	5.18±0.12a	nd	0.83±0.09a	6.17±0.24c	nd	0.43±0.00d	nd	nd
	YM122	28.36±3.58a	2.28±0.13b	4.42±0.38abc	nd	0.44±0.03ab	95.92±7.52b	13.87±0.00b	20.14±1.83c	nd	20.70±0.95d
6	DM068	24.83±4.63ab	nd	4.74±0.87ns	nd	2.56±0.24e	80.28±7.77a	nd	7.37±0.25bc	7.20±0.01c	237.80±34.52a
	JM0812	21.45±1.58b	nd	5.11±0.38ns	nd	6.44±0.11c	85.57±2.33a	nd	6.19±0.23c	11.38±0.85a	166.95±1.76b
	JM085	27.88±0.41a	nd	5.35±0.04ns	nd	2.90±0.06d	84.44±1.18a	nd	8.63±0.27ab	7.60±0.24c	244.58±1.39a
	UM054	20.62±1.15b	nd	4.93±0.30ns	nd	6.68±0.08abc	81.45±1.82a	nd	4.08±0.06d	4.74±0.10d	128.85±12.18c
	UM055	21.46±1.14b	nd	5.12±0.30ns	nd	6.99±0.21a	82.10±1.49a	nd	4.38±0.04d	4.87±0.18d	146.10±16.08bc
	VM095	21.15±0.30b	nd	5.02±0.14ns	nd	6.81±0.13ab	81.51±1.27a	nd	7.15±1.96bc	7.08±0.29c	122.43±12.39c
	VM096	20.93±0.01b	nd	4.83±0.00ns	nd	6.61±0.04bc	4.90±0.25b	nd	nd	nd	nd
	YM122	20.54±0.42b	nd	5.35±0.13ns	nd	6.97±0.06a	80.97±0.12a	nd	10.16±0.23a	10.52±0.26b	110.43±3.67c
12	DM068	22.43±2.35a	nd	5.22±0.49b	nd	3.55±0.25b	76.05±6.11ab	nd	13.26±2.06a	9.39±1.00a	457.32±47.71a
	JM0812	17.22±0.05b	nd	4.61±0.12c	nd	10.78±0.83a	71.05±0.09b	26.91±6.56a	7.47±1.36b	7.16±0.27bc	224.94±3.68c
	JM085	23.98±0.16a	nd	5.73±0.05a	nd	4.06±0.07b	81.43±3.10a	nd	5.41±1.73b	6.67±1.44c	346.14±3.78b
	UM054	17.65±0.35b	nd	4.74±0.22c	nd	10.44±0.77a	71.30±0.07b	10.19±0.00c	13.99±1.81a	9.58±0.18a	221.96±0.57c
	UM055	16.40±0.20b	nd	4.52±0.01c	nd	11.41±0.47a	72.26±1.95b	nd	12.41±0.00a	9.79±0.16a	198.83±2.00c
	VM095	16.77±0.03b	nd	4.43±0.01c	nd	11.13±0.25a	71.16±2.37b	28.48±0.00a	14.46±0.72a	9.12±0.02ab	192.12±0.68c
	VM096	16.33±0.23b	nd	4.45±0.05c	nd	11.00±0.56a	4.40±0.17c	1.04±0.27d	nd	nd	nd
	YM122	16.73±0.10b	nd	4.43±0.01c	nd	10.95±0.71a	72.09±1.94b	19.61±0.00b	14.74±0.53a	8.75±1.74abc	208.46±12.24c

Table 4.3 Continued

Incubation Time (h)	*Lb. pentosus* Strains	The capacity of raffinose utilisation, lactic acid, and SCFAs production by probiotic *Lb. pentosus* 8 strains									
		Sugar contents (mg/mL)				Lactic acid (mg/mL)	SCFAs (µmol/mL)				
		raffinose	D(+) glucose	galactose	D(-) fructose	Lactic acid	Acetic acid	Propionic acid	Butyric acid	Iso-butyric acid	n-valeric acid
24	DM068	22.29±1.38a	nd	5.83±0.35a	nd	5.35±0.24b	72.50±5.56d	nd	3.06±0.50e	9.22±0.51bc	21.13±2.60f
	JM0812	10.47±0.25b	nd	2.72±0.17b	nd	17.72±0.05a	86.56±0.83abc	22.43±3.43c	15.62±0.47b	10.00±0.20b	624.22±3.74a
	JM085	23.52±1.01a	nd	6.15±0.27a	nd	5.47±0.09b	78.77±4.52cd	29.49±2.79a	13.32±0.50c	15.55±2.33a	nd
	UM054	10.44±0.03b	nd	2.71±0.12b	nd	17.55±0.66a	79.15±2.31cd	26.69±0.97b	19.98±0.54a	7.49±0.60cd	563.41±5.16b
	UM055	10.49±0.07b	nd	2.66±0.02b	nd	17.82±0.29a	93.31±0.18ab	27.59±0.38b	14.23±0.39c	4.88±1.11e	473.33±27.26c
	VM095	10.37±0.20b	nd	2.81±0.08b	nd	17.55±0.31a	89.02±9.43abc	21.76±0.68c	9.68±0.43d	8.33±0.48bc	313.94±9.77d
	VM096	10.58±0.08b	nd	2.78±0.10b	nd	17.48±0.40a	98.49±8.37a	20.38±0.91c	8.58±0.21d	5.13±0.13e	nd
	YM122	10.30±0.00b	nd	2.62±0.00b	nd	17.93±0.23a	82.43±1.95bcd	19.26±0.38c	8.79±0.89d	5.64±0.09de	278.12±2.18e

Note: the mean values ± SD in the same column, in the same time of each strain with different small letters were significant different ($p<0.05$) ns= non significant, nd= non detectable

4.1.2.4 FOS utilisation

The capacity of FOS utilisation lactic acid and SCFAs production by *Lb. pentosus* 8 strains in MRS medium contains 2% FOS are summarized in Table 4.4. At the initial, sugar contents in culture medium was analysis by HPLC. It result found FOS in range between (5.78 ± 0.07 to 6.49 ± 0.05mg/mL), treharose (4.96 ± 1.92 to 6.14 ± 0.30 mg/mL), D (+) glucose (11.67 ± 2.38 to 12.95 ± 0.30 mg/mL), and D (-) fructose (13.13 ± 0.09 to 14.76 ± 0.11 mg/mL). Meanwhile, molecule of FOS was breakdown to monosaccharide glucose, and fructose from the preparation by autoclaving. However, disaccharide treharose came from molecule of glucose attach glucose in the culture media. The utilisation of FOS was significant different ($p < 0.05$) among strains at 24 h incubation. Never the less, FOS slightly decreased over time, treharose, D (+) glucose and fructose were decreased also. At 24 h incubation, the UM055, and UM054 were highest utilized FOS from 6.49 ± 0.05 to 5.02 ± 0.23 mg/mL and from 6.38 ± 0.04 to 5.24 ± 0.19 mg/mL. In addition, the amount of treharose, D (+) glucose and fructose were significant different ($p < 0.05$) among strains at 24 h incubation. Six of eight strains had high capacity of sugar utilisation except DM068 and JM085.

In the same time, whereas sugar decreased lactic acid was increasing by time due to bacteria consumption of sugar and produced organic acid. During 24 h incubation, 6 of 8 strains could produce high amount of lactic acid after first 6 h incubation except DM068 and JM085 strains. There were significant different ($p < 0.05$) highest produced amount of lactic acid between range 16.64 ± 1.10 to 17.54 ± 0.33 mg/mL at 24 h incubation. Acetic acid increased during time incubation, the concentration of propionic, butyric, and n-valeric acid were significant different ($p < 0.05$) among strains at 24 h incubation but iso-butyric did not detect. The high concentration of propionic acid was 60.28 ± 6.12µmol/mL and 56.06 ± 6.39 µmol/mL by UM054 and JM0812, respectively. However, high amount of butyric acid was produced by UM055 and YM122 (46.08 ± 0.25 µmol/mL and 42.85 ± 0.00 µmol/mL), and n-valeric acid was produced by YM122 (614.41 ± 71.85µmol/mL).

Table 4.4 The capacity of FOS utilisation, lactic acid, and SCFAs production by probiotic *Lb. pentosus* 8 strains in FOS-MRS medium 24 h incubation period.

Incubation Time (h)	*Lb. pentosus* Strains	The capacity of FOS utilisation, lactic acid, and SCFAs production by probiotic *Lb. pentosus* 8 strains									
		Sugar contents (mg/mL)				Lactic acid (mg/mL)	SCFAs (µmol/mL)				
		FOS	treharose	D(+) glucose	D(-) fructose	Lactic acid	Acetic acid	Propionic acid	Butyric acid	Iso-butyric acid	n-valeric acid
0	DM068	6.28±0.32ns	6.14±0.30ns	12.93±0.46ns	14.67±0.75ns	0.39±0.03b	45.10±0.58a	40.83±2.09c	19.80±1.05b	nd	362.72±5.21ab
	JM0812	5.81±0.76ns	5.63±0.33ns	12.36±1.11ns	13.89±1.24ns	0.60±0.00b	45.77±2.02a	64.22±0.45a	56.06±6.39a	nd	341.80±5.39ab
	JM085	5.79±0.93ns	4.96±1.92ns	11.67±2.38ns	14.03±1.39ns	0.47±0.15b	48.32±3.95a	50.07±5.32bc	21.14±0.62b	nd	391.18±0.00a
	UM054	6.38±0.04ns	6.00±0.11ns	12.95±0.30ns	14.70±0.29ns	0.62±0.05b	48.86±2.16a	60.94±5.39a	60.28±6.12a	nd	nd
	UM055	6.49±0.05ns	5.96±0.03ns	13.00±0.05ns	14.76±0.11ns	0.66±0.12b	48.08±0.42a	66.54±5.52a	19.28±0.06b	nd	nd
	VM095	5.99±0.30ns	5.61±0.21ns	12.30±0.34ns	13.86±0.48ns	0.67±0.04b	25.84±0.41b	65.42±2.56a	10.57±0.02c	nd	316.03±6.64b
	VM096	5.78±0.07ns	5.34±0.01ns	11.71±0.15ns	13.13±0.09ns	0.60±0.06b	27.06±0.90b	57.21±4.51ab	20.58±0.00b	nd	241.42±58.73c
	YM122	5.85±0.48ns	5.15±0.11ns	11.88±1.07ns	13.19±0.09ns	1.31±0.35a	28.40±1.78b	59.79±5.51ab	22.12±2.79b	nd	239.75±10.13c
6	DM068	5.63±0.21ns	5.08±0.01a	9.87±0.03ns	12.94±0.20ns	2.18±0.06b	116.46±2.38a	32.77±4.02b	19.80±1.05b	nd	253.28±38.37b
	JM0812	5.62±0.21ns	1.90±0.00cd	9.23±0.42ns	12.02±0.55ns	6.66±0.47a	80.47±0.11b	49.43±1.08a	56.06±6.39a	nd	nd
	JM085	5.58±0.34ns	5.00±0.06a	9.79±0.19ns	12.88±0.05ns	2.27±0.22b	115.90±3.46a	37.54±3.43b	21.14±0.62b	nd	495.09±95.61a
	UM054	5.55±0.01ns	1.83±0.04cd	9.18±0.30ns	12.00±0.19ns	6.93±0.21a	115.33±5.82a	51.48±4.39a	60.28±6.12a	nd	nd
	UM055	5.68±0.30ns	1.78±0.13d	9.41±0.73ns	12.16±1.26ns	7.18±1.15a	117.90±15.50a	50.21±6.59a	19.28±0.06b	nd	nd
	VM095	5.56±0.04ns	1.99±0.12bc	9.33±0.36ns	11.90±0.15ns	6.63±0.42a	114.75±6.43a	53.87±2.40a	10.57±0.02c	nd	nd
	VM096	5.47±0.14ns	2.14±0.05b	9.12±0.32ns	11.83±0.43ns	6.51±0.30a	116.61±3.69a	52.45±3.71a	20.58±0.00b	nd	nd
	YM122	5.45±0.30ns	1.73±0.03d	9.27±0.03ns	12.12±0.08ns	7.12±0.11a	117.54±1.56a	54.23±1.32a	22.12±2.79b	nd	nd
12	DM068	5.81±0.10ns	4.28±0.03a	10.31±0.05a	13.45±0.32a	3.53±0.13b	118.54±2.07a	37.77±1.39bc	19.80±1.05b	nd	568.02±41.57a
	JM0812	5.68±0.32ns	1.47±0.07c	8.03±0.91ab	10.65±1.23c	12.17±0.42a	88.33±7.71b	38.02±1.25bc	56.06±6.39a	nd	nd
	JM085	5.36±1.06ns	4.06±0.84ab	9.69±1.74ab	12.57±2.51b	3.29±0.57b	112.76±20.46a	43.70±9.70ab	21.14±0.62b	nd	333.99±59.23b
	UM054	5.74±0.12ns	1.52±0.09c	8.21±0.45ab	10.77±0.96c	11.49±0.33a	86.33±5.78b	47.67±7.97ab	60.28±6.12a	nd	202.16±0.00c
	UM055	5.75±0.17ns	1.54±0.01c	8.16±0.03ab	10.64±0.13c	11.78±0.32a	85.14±4.29b	28.00±4.15c	19.28±0.06b	nd	201.52±0.00c
	VM095	5.37±0.13ns	3.44±0.01b	9.42±1.58ab	11.10±2.16bc	11.25±1.56a	114.75±6.43a	53.87±2.40a	10.57±0.02c	nd	nd
	VM096	5.93±0.09ns	1.55±0.04c	7.78±0.22b	10.69±0.38c	12.31±0.49a	116.61±3.69a	52.45±3.71a	20.58±0.00b	nd	nd
	YM122	5.60±0.36ns	1.54±0.08c	8.04±0.42ab	10.33±0.49c	11.71±0.41a	117.54±1.56a	54.23±1.32a	22.12±2.79b	nd	nd

Table 4.4 Continued

Incubation Time (h)	*Lb. pentosus* Strains	The capacity of FOS utilisation, lactic acid, and SCFAs production by probiotic *Lb. pentosus* 8 strains									
		Sugar contents (mg/mL)				Lactic acid (mg/mL)	SCFAs (µmol/mL)				
		FOS	treharose	D(+) glucose	D(-) fructose	Lactic acid	Acetic acid	Propionic acid	Butyric acid	Iso-butyric acid	n-valeric acid
24	DM068	5.76±0.07ab	3.02±0.07a	10.39±0.30a	13.22±0.16a	4.95±0.18b	110.23±3.02b	19.80±1.05b	0.43±0.00f	nd	354.28±27.74c
	JM0812	5.10±0.12c	1.42±0.01b	6.09±0.11b	7.95±0.01b	17.32±0.37a	79.60±2.66c	56.06±6.39a	3.91±0.00e	nd	78.89±0.00d
	JM085	5.87±0.06a	3.02±0.01a	10.55±0.27a	13.80±0.09a	5.12±0.28b	113.93±5.65ab	21.14±0.62b	0.43±0.00	nd	394.51±6.55c
	UM054	5.24±0.19bc	1.46±0.05b	5.96±0.21b	8.00±0.29b	17.34±0.76a	80.59±3.30c	60.28±6.12a	nd	nd	nd
	UM055	5.02±0.23c	1.51±0.03b	5.91±0.18b	8.01±0.08b	17.54±0.33a	120.58±6.42a	19.28±0.06b	46.08±0.25a	nd	588.85±36.31a
	VM095	5.20±0.19bc	1.47±0.08b	5.92±0.30b	8.06±0.14b	17.54±0.41a	70.71±1.98c	10.57±0.02c	40.35±0.00c	nd	133.68±19.12d
	VM096	4.94±0.47c	1.41±0.12b	5.65±0.56b	7.69±0.70b	16.64±1.10a	121.05±3.58a	20.58±0.00b	25.46±1.95d	nd	475.53±37.15b
	YM122	5.14±0.22c	1.49±0.13b	5.88±0.30b	7.93±0.55b	17.23±1.05a	118.16±4.34ab	22.12±2.79b	42.85±0.00b	nd	614.41±71.85a

Note: the mean values ± SD in the same column, with the same parameter in the same period of each strain with different small letters were significant different ($p<0.05$) ns= non significant, nd= non detectable

Sugar utilisation from different carbohydrates (glucose, lactose, raffinose, and FOS) as a carbon source in MRS media and inoculated with probiotic *Lb. pentosus* 8 strains in 24 h incubation are summarized in Table 4.5. The sugar utilisation was significant different ($p < 0.05$) by time incubation for each strain in the culture medium. The 8 strains had a high capacity to use glucose in culture media more than 90 % (rang 94.49 - 94.83 %). The consumption of glucose in medium greater happened at 12 h due to the growth of *Lb. pentosus* maintained in the exponential phase and reached maximium OD.

Never the less, the capacity lactose utilisation in culture medium was lower than glucose in range 1.22 - 68.34 %. The 6 strains had a capacity of lactose utilisation more than 50 % that were VM096, YM122, UM054, UM055, VM095 and JM0182 by 68.34, 67.54, 59.55, 57.69, 57.55 and 53.21 %, respectively.

The capacity of raffinose utilisation in culture medium was lower than glucose in rang between 6.38 - 68.78%. It can be seen only 3 of 8 strains had a capacity of raffinose utilisation more than 50% were UM055, YM122, and JM0182 by 63.78, 63.68, and 51.07%, respectively.

In addition, the capacity of FOS utilisation in culture meduim was lower than glucose in highest amount 22.65 % by UM055 strain.

Table 4.5 Sugar utilisation from different carbohydrates (glucose, lactose, raffinose, and FOS) as a carbon source in modified-MRS media and inoculated with probiotic *Lb. pentosus* 8 strains in 24 h incubation.

Incubation time (h)	Sugar utilisation by *Lb. pentosus* strains							
	Glucose contents (mg mL^{-1})							
	DM068	JM0812	JM085	UM054	UM055	VM095	VM096	YM122
0	17.18±0.05A	17.31±0.38A	17.61±1.66A	17.32±0.26A	17.34±0.19A	17.26±0.19A	16.91±0.19A	16.69±0.29A
6	17.04±0.98A	16.98±1.04A	17.61±0.48A	17.11±0.12A	16.93±0.12A	17.06±0.00A	17.16±0.15A	17.14±0.14A
12	9.36±0.01B	9.39±0.08B	9.18±0.02B	9.53±0.24B	9.09±0.39B	9.45±0.01B	9.24±0.03B	9.53±0.09B
24	0.94±0.01C	0.92±0.00C	0.91±0.01C	0.93±0.00C	0.91±0.00C	0.92±0.01C	0.91±0.01C	0.92±0.01C
Substrate conversion (%)	94.53	94.69	94.83	94.63	94.75	94.67	94.68	94.49
Incubation time (h)	Lactose contents (mg mL^{-1})							
	DM068	JM0812	JM085	UM054	UM055	VM095	VM096	YM122
0	25.67±1.75A	28.32±2.57A	28.90±0.52A	26.85±0.15A	27.75±1.64A	28.95±2.11A	36.54±1.59A	34.50±0.33A
6	25.05±1.11A	26.58±1.32A	28.47±1.78A	24.49±0.77B	25.32±0.42A	25.53±0.85B	25.66±1.71B	25.74±1.48B
12	24.82±2.91A	19.10±0.83B	26.96±1.47A	18.21±0.91C	18.78±0.91B	19.29±0.42C	19.74±1.21C	19.43±2.01C
24	24.45±0.91A	13.25±0.71C	22.55±0.54B	10.86±0.08D	11.74±0.61C	12.29±0.77D	11.57±0.49D	11.20±0.06D
Substrate conversion (%)	1.22	53.21	21.97	59.55	57.69	57.55	68.34	67.54
Incubation time (h)	Raffinose contents (mg mL^{-1})							
	DM068	JM0812	JM085	UM054	UM055	VM095	VM096	YM122
0	23.81±5.02A	21.40±3.49A	26.46±1.36A	18.02±0.86B	28.96±2.52A	17.80±1.63B	18.53±0.04B	28.36±3.58A
6	24.83±4.63A	21.45±1.58A	27.88±0.41A	20.62±1.15A	21.46±1.14B	21.15±0.30A	20.93±0.01A	20.54±0.42B
12	22.43±2.35A	17.22±0.05A	23.98±0.16B	17.65±0.35B	16.40±0.20C	16.77±0.03B	16.33±0.23C	16.73±0.10C
24	22.29±1.38A	10.47±0.25B	23.52±1.01B	10.44±0.03C	10.49±0.07D	10.37±0.20C	10.58±0.08D	10.30±0.00D
Substrate conversion (%)	6.38	51.07	11.11	42.06	63.78	41.74	42.90	63.68
Incubation time (h)	FOS contents (mg mL^{-1})							
	DM068	JM0812	JM085	UM054	UM055	VM095	VM096	YM122
0	6.28±0.32A	5.81±0.76A	5.79±0.93A	6.38±0.04A	6.49±0.05A	5.99±0.30A	5.78±0.07A	5.85±0.48A
6	5.63±0.21B	5.62±0.21A	5.58±0.34A	5.55±0.01BC	5.68±0.30B	5.56±0.04AB	5.47±0.14AB	5.45±0.30A
12	5.81±0.10AB	5.68±0.32A	5.36±1.06A	5.74±0.12B	5.75±0.17B	5.37±0.13B	5.93±0.09A	5.60±0.36A
24	5.76±0.07AB	5.10±0.12A	5.87±0.06A	5.24±0.19C	5.02±0.23C	5.20±0.19B	4.94±0.47B	5.14±0.22A
Substrate conversion (%)	8.28	12.22	-1.38	17.87	22.65	13.19	14.53	12.13

Note: The mean value ± SD with the same parameter for each strain, with different capital letters in the same row were significant different ($p<0.05$).

4.2 Screening for BSH activity

Cholesterol reducing is a health-promoting characteristic, the idea of selection of probiotic strains. However, cholesterol reducing activity of lactobacilli strain happened by several macchanisms. One of mechanisms through BSH activity has been associated reduction of cholesterol (Corzo and Gilliland, 1999; Liong and Shah, 2005a; Begley et al., 2006). In human, bile acids are synthesized from cholesterol and conjugated to either glycine or taurine in the liver then pass into the intestine, whereas amino acid maybe hydrolyzed from these conjugated bile acid by bacterial enzymes known as conjugated BSH, which expressed by gastrointestinal bacteria of several genera. When BSH-producing lactobacilli were streaked out on MRS plates containing 0.5% TDCA, the taurine-conjugated bile acid was deconjugated, producing deoxycholic acid (Mahrous, 2011).

In this study, 8 strains of the probiotic *Lb. pentosus* were screened for BSH activity by plate assay technique. The cell suspensions of overnight cultures were spotted 10 µL on sterile filter disces (diameter 0.7 cm) and are placed on MRS agar with 0.5% (w/v) TDCA and incubated anaerobically at 37 ^{o}C for 72 h. The deconjugation activity of *Lb. pentosus* strains was presented in Figure 4.6 B-I (disc No. 1-3). The amounts of deoxycholic acid precipitated around disc and diffused into the surrounding medium. Howevre, a positive control disc on MRS agar without 0.5% (w/v) TDCA did not found a precipitated zone around disc. However, the disc (No. 4) without spotted cell suspension also did not found a precipitated zone. All strains exhibited BSH activities as demonstrated by precipitation zones ($p < 0.05$) with diameters between 8.83 ± 0.75 mm to 10.17 ± 0.41 mm (Table 4.6). The greatest precipitation zone was found in *Lb. pentosus* VM 096 followed by VM095, YM122, JM085, and UM055 (10.17 ± 0.41, 9.92 ± 0.58, 9.83 ± 0.41, 9.83 ± 0.41, and 9.67 ± 0.52 mm, respectively) and the strain DM068 had the lowest precipitation of 8.83 ± 0.75 mm. The BSH-positive lactobacilli can be grouped in 3 classes based on the diameters of the precipitation zones. The precipitation zone up to 10 mm was demonstrated by low BSH activity; if the precipitation zone up 11 to 15 mm was demonstrated by medium; the precipitation zone is greater than 16 mm was demonstrated by high BSH activity (Mathara *et al.*, 2008). From the results, this study demonstrated that the 8 *Lb. pentosus* strains showed low BSH activity due to the precipitation zone surrounding the disces up to 10 mm.

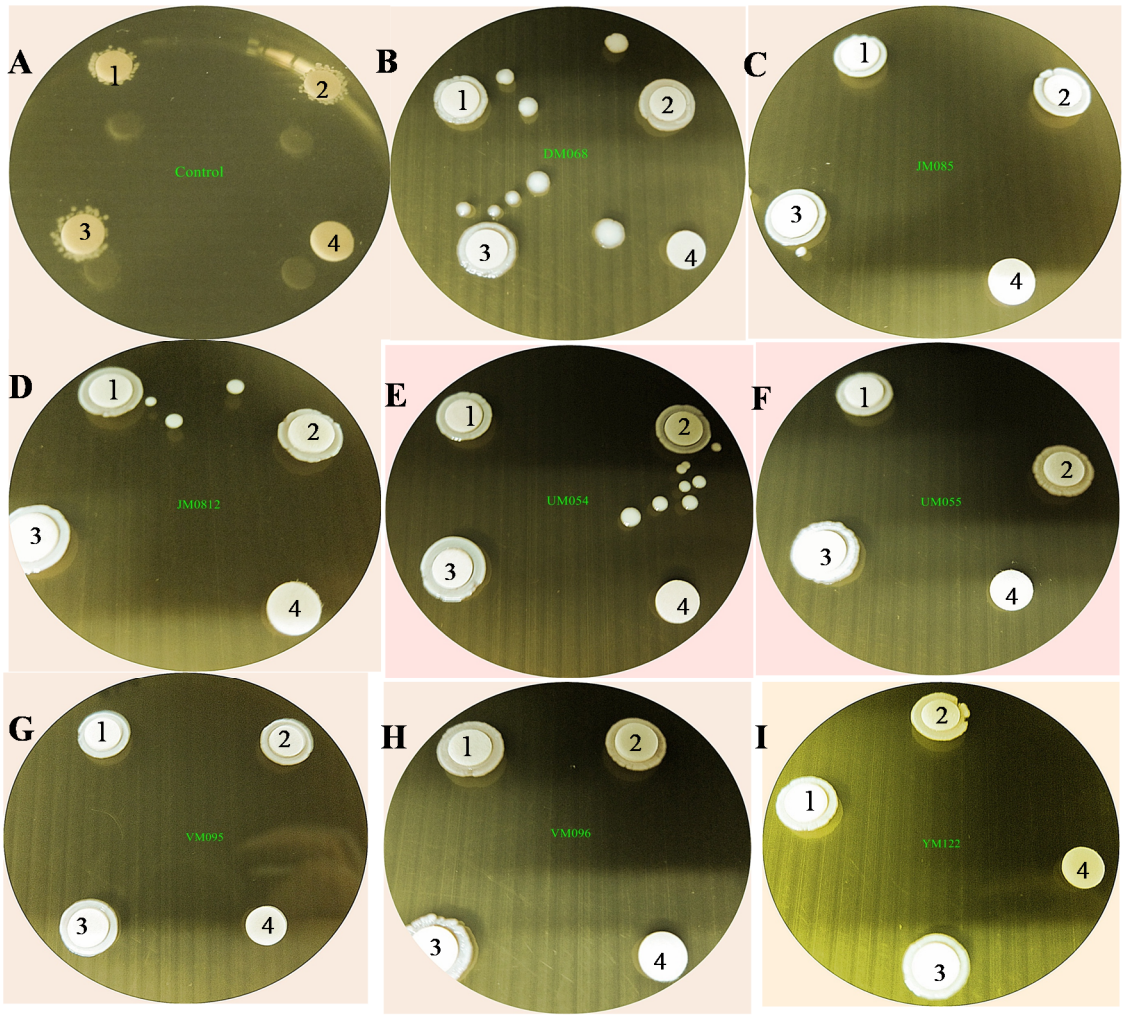

Figure 4.6 Characteristic of BSH activity by probiotic 8 *Lb. pentosus* strains on tested medium. The letters A was a control (MRS medium without 0.5% (w/v) TDCA as positive control), B-I were BSH activity by DM068, JM0812, JM085, UM054, UM055, VM095, VM096, YM122, respectively on MRS medium with 0.5% (w/v) TDCA. The sterile filter disces spotted with 10 µL cell suspensions of each strain (No. 1-3), and without spotted cell suspensions (No. 4) as a negative control.

Table 4.6 BSH activity of probiotic *Lb. pentosus* strains

Lb. pentosus strains	Diameter of precipitation zone (mm.)
DM068	8.83±0.75c
JM0812	9.33±0.52bc
JM085	9.83±0.41ab
UM054	9.33±0.51bc
UM055	9.67±0.52ab
VM095	9.92±0.58ab
VM096	10.17±0.41a
YM122	9.83±0.41ab

Notes: values are the mean ± SD of three independent experiments performed in duplicates. Different letter followed mean values in the same column indicate significant different ($p<0.05$) between the treatments ($n=6$).

These results supported other published work that tested the activity of BSH for some probiotic lactobacilli and all tested strains gave a BSH-positive. Mahrous (2011) reported that *Lb. acidophilus* P106 had greater precipitation followed by *Lb. acidophilus* P110, *Lb. plantarum* P164, and *Lb. pentosus* P191, respectively. However, Sieladie et al. (2011) found that 15 isolates of *Lb. plantarum* from raw cow milk displayed BSH activity and 4 isolates exhibited BSH activity by demonstrated precipitation zone diameter 12 to15 mm and 11 expressing precipitation zone diameter greater than 15 mm. However, Pereira et al. (2003) reported that only 5 of 14 strains of lactobacilli had shown positive BSH activity with precipitation zones different in size. The *Lb. fermentum* KC5b and *Lb. plantarum* NDV^R strain displayed the largest zones. In addition, Silirun et al. (2010) found that 4 of 16 *lactobacillus sp.* (TGCM 15, TGCM 33, SC 359 and LCC 150) displayed BSH activity by providing the precipitation zone around colonies on plate assay. The TGCM 15 and TGCM 33 strain were identified as *Lb. plantarum*. The results suggest that the BSH ability supported the mechanism for the *in vitro* lowering of cholesterol of the cells (Parvez et al., 2006; Kim et al., 2008).

Bile salt deconjugation is an important characteristic as it could play a role in maintaining the equilibrium of the gut microflora in reducing serum cholesterol and in

the production of a detergent shock protein that enables *lactobacillus sp.* to survive exposure to bile (Corzo and Gilliland, 1999). The high BSH activity of *lactobacillus* might have some role in the reduction of the serum cholesterol level. Bile excretion is a major route of eliminating cholesterol from the body, as well as one of the important pathways of cholesterol metabolism (Agaliya and Jeevaratnam, 2012). Liong and Shah (2005a) explained that BSH secreted from lactobacilli were able to catalyze the hydrolysis of glycine-conjuagted bile or taurine-conjuagted bile into amino acid residues and free bile salts (free cholic acids). Free bile salts are less soluble than conjugated bile salts (glycine-conjuagted bile or taurine-conjuagted bile), providing lower absorption in the intestinal lumen and excretion into feces, whereas amino acid group was reabsorbed into intestinal tract. Thus, free bile salts (deconjugation bile acids) can reduce serum cholesterol level by increasing the formations of new bile acids instead of those free bile acids from the enterohepatic circulation and cholesterol act as a precursos of bile salts (Ooi and Liong, 2010; Sirilun et al., 2010).

4.3 *In vitro* cholesterol binding activity

The 8 *Lb. pentosus* strains were measured for their ability to reduce cholesterol *in-vitro* in the presence of bile salts as illustrated in Figure 4.7. The amount of cholesterol reduced ranged 20.31 to 31.30 μg/mL (29.01 ± 1.38 to 44.71 ± 1.33 % reduction). All strains showed a significant ($p < 0.05$) reduction in cholesterol concentration in culture broth. The highest percentage (44.71 ± 1.33%) of cholesterol removed was recorded in strain VM096. The ability to reduce cholesterol of the strains was in the order of UM055, VM095, YM122, and UM054 (41.63 ± 1.12%, 39.34 ± 1.48%, 35.68 ± 1.75%, and 35.19 ± 1.00 %, respectively). The JM085 strain had the lowest ability to reduce cholesterol by 29.01 ± 1.38% reduction.

These results supported the other publish work that observed the ability to reduce cholesterol *in-vitro*. Hyeong et al, (2004) found that *lactobacillus* strains could remove 31.5 to 58.5% cholesterol in the MRS medium with 0.3% oxgall. However, Ramasamy et al (2009) also reported that 12 *lactobacillus* strains were varying able to remove 26.74 to 85.41% cholesterol among the strains. Sirilun et al (2010) demonstrated that the 4 *Lb. plantarum* isolated from food origins were considered as the effective probiotics with cholesterol-lowering property capable of reducing 25.41 to

81.46% from the MRS medium with 0.3% oxgall after 24 h incubation. Several work indicated that *lactobacillus* species were able to reduce cholesterol *in-vitro* via several mechanisms such as an uptake or assimilation of cholesterol by bacteria strains, cholesterol adherence to the bacteria cells wall or its incorporation into bacterial cells, including bile salt deconjugation (Gilliland et al., 1985; Liong and Shah, 2005; Silirun, 2010; Madani et al, 2013). In addition, it can be suggested that the BSH ability of microorganism supported the mechanism for the *in vitro* cholesterol binding of the cells (Parvez et al., 2006; Kim et al., 2008). This corresponded to the report that deconjugated bile salts can co-precipitate in acidic environment at pH lower than 5.5 (Klaver and Van der Meer, 1993; Mathara et al., 2008).

Hypercholesterol is a risk factor for cardiovascular diseases, which is a leading cause of death for human. A 1% reduction in serum cholesterol is estimated to result in 2-3% reduction in the risk of coronary disease. It is suggested that intestinal lactobacilli may reduce serum cholesterol level through bacterial assimilation of cholesterol in the intestine and deconjugation of bile salts. SCFAs produced by lactobacilli may also inhibit hepatic cholesterol systhesis and distribution of cholesterol in the plasma and liver (Collado, 2009)

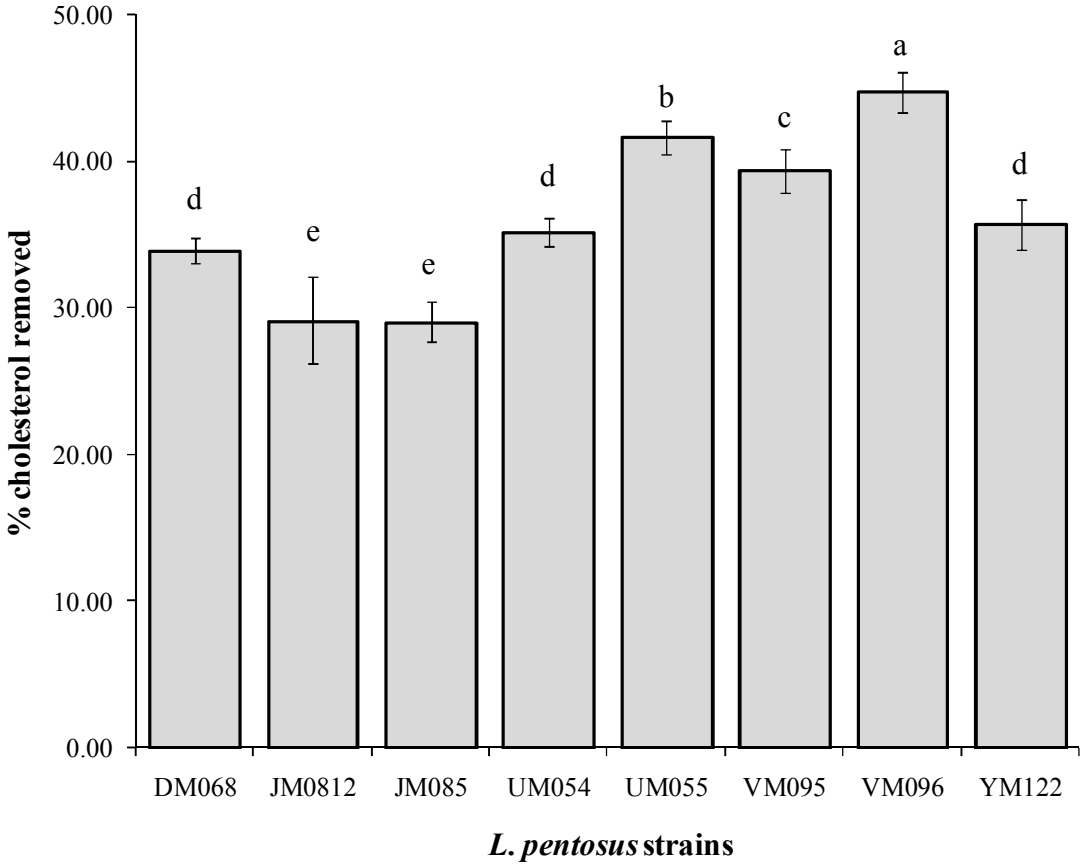

Figure 4.7 Percentage of cholesterol removed by 8 probiotic *Lb. pentosus* strains after 24 h incubation. The error bars indicated the standard deviation (SD) and different superscript letters showed significant different means ($p < 0.05$), n=3.

4.4 ZEA binding ability by *Lb. pentosus* strains in phosphate buffer

ZEA is mycotoxin, which is considered to be causes in economic loss and serious health problems humans and animals as well by contaminated in various cereals crop such as soybean, rice, corn, wheat and grain crops. In Thailand, the report about ZEA contamination in food products had little information. The method to reduce ZEA contamination have been considerable attention but some safe and efficient methods are not practical and too expensive. Several researches found that LAB were able to remove mycotoxin by binding process (Jespersen, 2006) and Zinedine et al, 2007). Therefore, our study was determined of the efficiency of ZEA binding by probiotic 8 *Lb. pentosus* strains which is a first report in Thailand.

The binding ability to ZEA of 8 strains of probiotic *Lb. pentosus* was investigated. Five-milligram dry weight of each strain was tested with various concentration levels of ZEA toxin in sodium acetate buffer solution pH 5.0. The ZEA amount recovered from supernatant indicated the binding ability of the tested strains. The low percentage of toxin remaining indicated high efficiency in adsorption of ZEA into bacterial cells. The binding ability to ZEA of test strains was shown in Figure 4.8 and Table 4.7.

As illustrated in the Figure 4.8, from the initial concentration level of ZEA at 1.10 µg/mL, the result showed 8 strains could bind ZEA more than 40%. All 8 strains were able to bind ZEA between 0.40 ± 0.04 to 0.55 ± 0.01 µg/mL (about 36.18 ± 3.48 % to 49.90 ± 0.74%). The strain *Lb. pentosus* JM085 had the greatest binding ability whereas YM122 had the lowest of binding ability. However, the concentration level of ZEA at 5.51 µg/mL, the ability of test strains to bind ZEA were between 1.35 ± 0.06 to 2.05 ± 0.03 µg/mL (about 24.46 ± 1.10 % to 37.19 ± 0.62%). At the higher initial concentration level of ZEA (23.08 µg/mL) the ability of ZEA was in the range of 7.70 ± 0.11 to10.97 ± 2.37 µg/mL (33.37 ± 0.47 to 47.50 ± 10.27 %). The strains with > 40% of ZEA binding were JM085, UM054 and DM068 (47.50%, 43.18%, and 42.78%, respectively) as present in Table 4.7.

The results of binding tests at the concentration ZEA 51.79 µg/mL found 3 strains were highest ability to bind ZEA reach to 70% that DM068, VM096 and UM055 could bind 75.17%, 70.38% and 70.00%, respectively. However, at the highest concentration level of ZEA (75.70 µg/mL), all strains of *Lb. pentosus* could bind ZEA in between 44.93 ± 16.92 to 62.12 ± 0.61 µg/mL (60.15 ± 22.56% to 83.17 ± 0.83%). We found that 3 strains had capability to bind ZEA higher than 80%. The strain that could greatest detoxify ZEA was UM0812 (83.17%) followed by JM054 (82.02%) and UM055 (81.69%). The results found that the binding abilities of the tested strains were significant different ($p < 0.05$) at various concentration levels of ZEA in buffer solution as shown in Table 4.7. At the highest concentration level of ZEA (74.70 µg/mL), 3 strains of *Lb. pentosus* (JM0812, UM054, and UM055) absorbed more than 80% of ZEA. Never the less, the concentration at 51.79 µg/mL of ZEA, the strains DM068, VM096, and UM055 had the binding capability higher than 70% up. Never the less, testing at the lower level of ZEA concentration (1.10-23.08 µg/mL), the best strain of *Lb. pentosus* could eliminate not more than 50% of ZEA. Regarding to % ZEA binding,

it can be seen that an opposite result of the lowest ZEA concentration level (1.10 μg/mL) was observed comparing to the highest levels (23.08-74.70 μg/mL). Probably the concentration at this point was too low for binding capacity and easy to get error; as a consequence, it was out of the standard curve range.

These results indicated that the binding efficiency of *Lb. pentosus* strains test depended greatly on the initial concentration of toxin in buffer solution. This finding is similar to a report of Fuchs et al (2008) which studied the binding of patulin and ochratoxin from liquid medium by LAB that the eliminations of mycotoxins from liquid medium were increased when the concentrations of the mycotoxins are higher. El-Nezami et al. (2002) stated that *Lb. rhamnosus GG* and *Lb. rhamnosus* LC-705 had ability to bind ZEA and its derivative (α-zearalenone) in liquid medium 38% and 46%, respectively. In contrast, Joannis-cassan et al (2011), who reported that mycotoxin binding by yeasts or yeast cell walls (levels 5 mg/mL), for ZEA, a decrease in the adsorption (%) was noted with the increasing initial concentration whereas AFB1 and OTA were differed with the type and initial concentration of mycotoxin.

The mechanism of mycotoxin binding by LAB has not been clearly described. LAB is gram-positive bacteria. Their thick cell wall consists of many layers of peptidoglycan protein and other components such as teichoic acid (TA), lipoteichoic acid (LTA) and polysaccharide (Delcour et al., 1999). A hypothesis stated that mycotoxin-binding positions were occurred at bacterial cell walls (Zinedine et al., 2007) and found that mycotoxin could be attached by teichoic acid and polysaccharide more than peptidoglycan (Shetty and Jespersen, 2006). These results concluded that binding ability of ZEA by the probiotic bacteria *Lb. pentosus* strains depend on the initial concentration levels of ZEA standard in the buffer solution test.

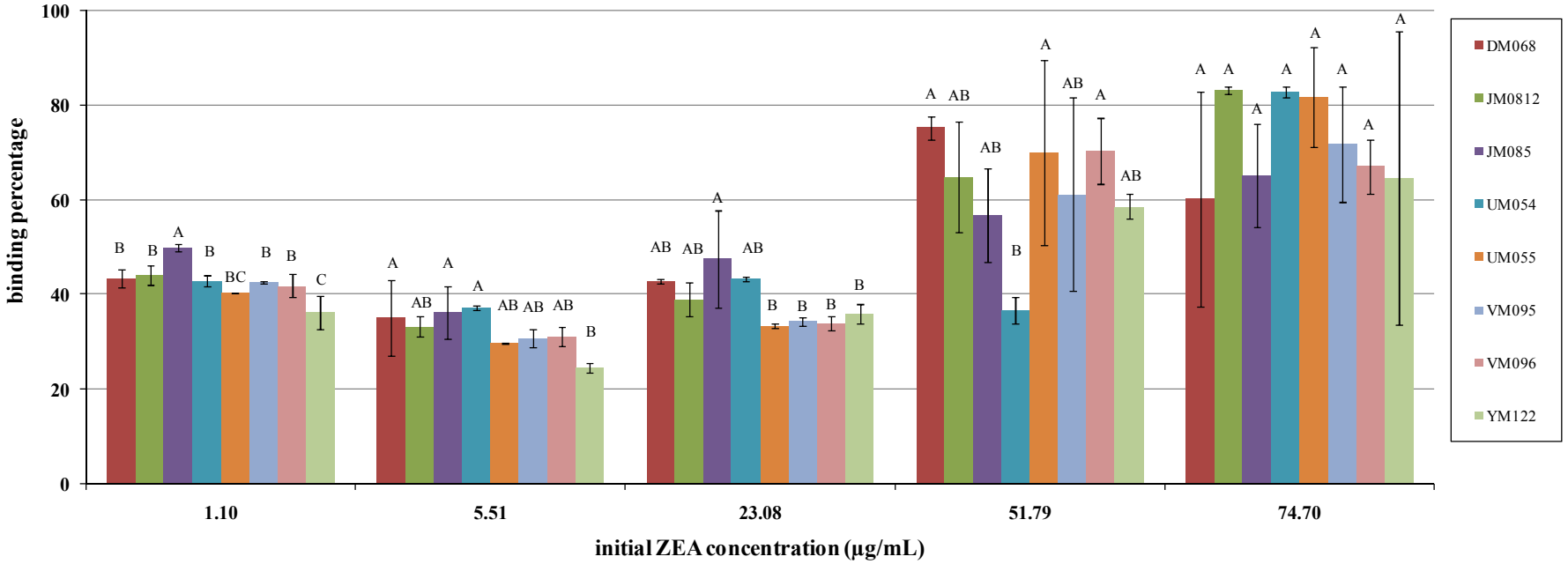

Figure 4.8 The binding ability (%) of ZEA by *Lb. pentosus* strains at 5 levels of ZEA concentration in 0.05 M sodium acetate buffer (pH 5.0). The error bars indicated the standard deviation (SD), n=2

Table 4.7 The ZEA binding ability of *Lb. pentosus* strains in buffer solution pH 5.0, ZEA Remaining concentration (C, remaining; μg/ml) and amout of ZEA adsorbtion (C, adsorbtion; μg/ml), and percentage of ZEA binding (%) at each initial toxin concentration.

Initial concentration of ZEA std (μg/ml)	Bio-adsorption assayed of *Lb. pentosus* strains							
	C, remaining (μg/ml)							
	DM068	JM0812	JM085	UM054	UM055	VM095	VM096	YM122
1.10	0.62±0.02Bb	0.62±0.02Bb	0.55±0.01Cc	0.63±0.01Bd	0.66±0.00ABc	0.63±0.03Bc	0.64±0.03Bc	0.70±0.04Ac
5.51	3.58±0.44Bb	3.68±0.31ABb	3.52±0.31Bc	3.46±0.03Bc	3.87±0.01ABc	3.82±0.11ABc	3.80±0.11ABc	4.16±0.06Ac
23.08	13.21±0.14ABab	14.09±2.37Aa	12.12±2.37Bbc	13.12±0.12ABb	15.38±0.11Aa	15.17±0.34Aabc	15.25±0.34Ab	14.78±0.48Ac
51.79	12.86±1.27Bab	18.23±5.14ABa	22.38±5.14ABab	32.79±1.46Aa	15.55±10.13Ba	20.14±3.61ABab	15.34±3.61Bb	21.46±1.37ABb
74.70	29.76±16.92Aa	12.57±8.11Aa	26.09±8.11Aa	12.87±0.80Ab	13.68±7.90Ab	21.15±4.21Aa	24.64±4.21Aa	26.52±23.12Aa
Initial concentration of ZEA std (μg/ml)	C, adsorption (μg/ml)							
	DM068	JM0812	JM085	UM054	UM055	VM095	VM096	YM122
1.10	0.48±0.02Bb	0.49±0.02Bd	0.55±0.01Ac	0.47±0.01Bd	0.44±0.00Cc	0.47±0.00Bc	0.46±0.03Bd	0.40±0.04Cc
5.51	1.93±0.44Ab	1.83±0.12ABb	1.99±0.31Ac	2.05±0.03Ad	1.64±0.01ABc	1.69±0.10ABc	1.71±0.11ABcd	1.35±0.06Bc
23.08	9.88±0.14ABb	8.99±0.81ABc	10.97±2.37Ac	9.97±0.12ABc	7.70±0.11Bc	7.92±0.20Bc	7.83±0.34Bc	8.30±0.48Bbc
51.79	38.93±1.27Aa	33.56±6.08ABb	29.41±5.14ABb	19.00±1.46Bb	36.24±10.13Ab	31.65±10.62ABb	36.45±3.61Ab	30.33±1.37Aab
74.70	44.93±16.92Aa	62.12±0.61Aa	48.61±8.11Aa	61.83±0.80Aa	61.02±7.90Aa	53.54±9.16Aa	50.06±4.21Aa	48.17±23.12Aa
Initial concentration of ZEA std (μg/ml)	ZEA Binding (%)							
	DM068	JM0812	JM085	UM054	UM055	VM095	VM096	YM122
1.10	43.37±1.95Bb	44.06±2.12Bc	49.90±0.74Aab	42.86±1.13Bb	40.27±0.01BCb	42.55±0.22Bbc	41.86±2.32Bb	36.18±3.48Cab
5.51	35.07±8.05Ab	33.22±2.21ABc	36.15±5.66Ab	37.19±0.62Ac	29.74±0.19ABb	30.68±1.86ABc	31.07±2.01ABb	24.46±1.10Bb
23.08	42.78±0.59ABb	38.97±3.50ABc	47.50±10.27Aab	43.18±0.53ABb	33.37±0.47Bb	34.29±0.86Bbc	33.92±1.47Bb	35.97±2.09Bab
51.79	75.17±2.44Aa	64.81±11.74ABb	56.78±9.93ABab	36.68±2.83Bc	69.98±19.56Aa	61.11±20.50ABab	70.38±6.97Aa	58.57±2.64ABab
74.70	60.15±22.65Aab	83.17±0.82Aa	65.08±10.85Aa	82.78±1.07Aa	81.69±10.58Aa	71.68±12.27Aa	67.02±5.63Aa	64.49±30.96Aa

Note: Values are means ± SD, n=2. Data with different capital letters in the same row and different small letters in the same column were significant different ($p<0.05$).

4.5 Adhesion ability

Probiotics are believed to temporarily colonise the intestine by adherence to intestinal surfaces. The adhesion ability can give information about the possibility of probiotics to colonise and modulate the host immune system. Several mechanisms were reported about the adhesion of microorganisms to intestinal epithelial cells. Cell hydrophobicity is one of factors that may contribute to adhesion of bacterial cells to host tissues (Savage,1992; Ram et al., 2003). This property is an advantage and importance for bacterial maintenance in the human gastrointestinal tract. Therefore, the adhesion ability of bacteria to intestinal cells has been considered as one of the selection criteria for probiotic strains (Salminen et al., 1996).

Eight strains of probiotic *Lb. pentosus* were compared for the *in vitro* cell surface hydrophobicity by determining bacterial adhesion to n-hexadecane. The assay method was modified from Rosenberg et al (1980). As a result of this study, cell surface hydrophobicity values were between 6.24% to 8.20% among bacteria tested as shown in Figure 4.9.

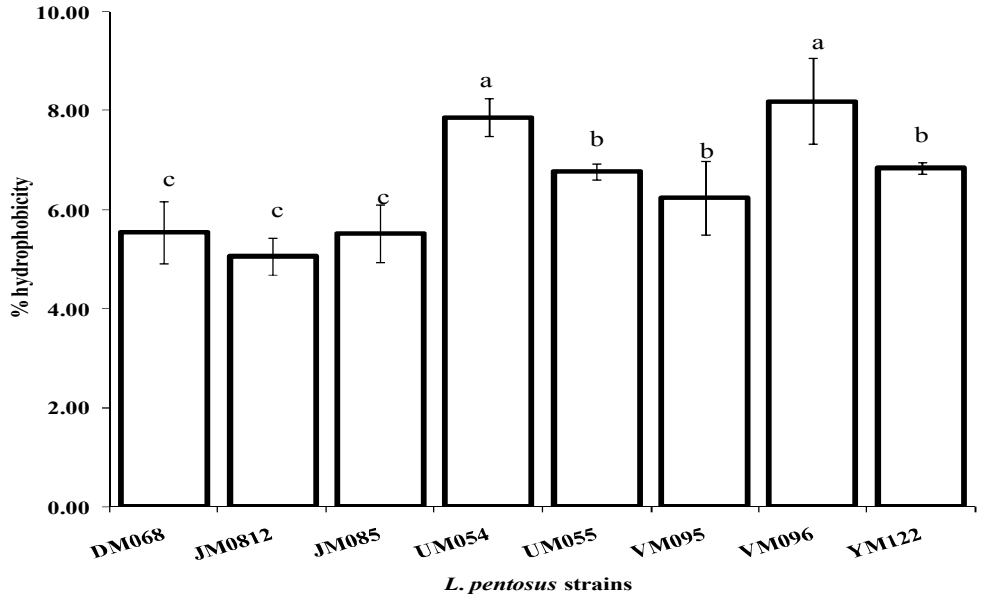

Figure 4.9 Cell surface hydrophobicity of 8 *Lb. pentosus* strains. The values are Mean ± SD of 3independent experiments performed in duplicates. The error bars indicate the standard deviation (SD) and different superscript letters are significant different ($p < 0.05$), $n=6$.

The results showed that the high value surface hydrophobicity of 8.20% and 7.87% were found for the strains VM096 and UM054, followed by the strains UM055, YM122, and VM095 were 6.85%, 6.77%, and 6.24%, respectively whereas strains DM068, JM0812, and JM085 were shown to have low value for hydrophobicity.

The partitioning of cells between water and hexadecane results from hydrophobic interactions between microorganisms and hydrocarbon. The percentages of adhesion to hexadecane of the strains indicate their surface hydrophobicity (Ly et al., 2010). Probiotic strains with high surface hydrophobicity might exist due to bacterial surface composition and structure. However, Schillinger et al. (2005) found that were between 2% to 94%. Strains of *Lb. acidophilus* tended to exhibit higher hydrophobicity values compared to *Lb. casei* strains and *Lb. rhamnosus* GG. The strains with a high hydrophobicity generally adhered to HT29 MTX cells at a high level but and strain with extremely low hydrophobicity of 2% was also able to adhere HT29 MTX cells at 40%. Hydrophbicity may be helpful for adhesion, but it is obviously not a prerequisite for a strong adherence capacity. Agaliya and Jeevaratnam (2012) concluded that adhesion is a complex process involving non-specific (hydrophobicity) and specific ligand-receptor mechanisms. The determination of microbial adhesion to hexadecane as a way to estimate the ability of a strain to adhere to epithelial cells is a valid qualitative phenomenological approach (Kiely and Olson, 2000). Adherence of bacterial cells is usually related to cell surface characteristics. Cell surface hydrophobicity is a non-specific interaction between microbial cells and host. The initial interaction may be weak, often reversible and precedes subsequent adhesion processes mediated by more specific mechanisms involving cell surface proteins and lipoteichoic acids (Rojas et al., 2002; Ross and Jonsson, 2002). The high hydrophobicity value of microorganism are usually associated with the presence of fibrillar structures on the cell surface and specific cell wall proteins, also cell surface hydrophobicity was related to cell age (the exponential growth phase) (Wang and Han, 2007).

Part II The use of probiotic *Lb. pentosus* as starter culture for probiotic soya beverage production.

The results from the previous section showed that the potential 3 strains of *Lb. pentosus* to be used as starter culture for soya beverage production were *Lb. pentosus* VM095, VM096, and YM122. The soya milk fermentation with a single

culture of each strain was determined at 2 h intervals over 24 h of fermentation. The pH change, the total acid production, bacterial enumeration, sugar and SCFA production by 3 *Lb. pentosus* strains in soya milk were observed.

4.6 Fermented soya milk characteristic

4.6.1 Enumeration of *Lb. pentosus* strains

Table 4.8 Enumeration of *Lb. pentosus* strains in fermented soya milk at 2 h intervals over 24 h of fermentation.

Incubation time (h)	Enumeration of *Lb. pentosus* (logCFU/ mL)		
	VM095	VM096	YM122
0	7.29±0.18b	7.43±0.14ab	7.70±0.17a
2	7.94±0.04ns	7.95±0.06ns	8.02±0.02ns
4	8.10±0.01b	8.13±0.05b	8.26±0.03a
6	9.34±0.06a	9.11±0.07a	9.18±0.11b
8	9.43±0.09a	9.25±0.06ab	9.42±0.10b
10	9.47±0.14ns	9.45±0.13ns	9.46±0.18ns
12	9.88±0.07ns	9.75±0.17ns	9.74±0.12ns
14	9.74±0.12a	9.50±0.04b	9.38±0.26b
16	9.93±0.11ns	9.98±0.06ns	9.97±0.05ns
18	10.15±0.06b	9.41±0.10c	10.32±0.01a
20	9.37±0.14ns	9.41±0.10ns	9.36±0.10ns
22	9.20±0.15ns	9.35±0.12ns	9.36±0.10ns
24	9.26±0.57ns	9.27±0.33ns	9.71±0.11ns

Note: Values are means±SD, n= 3. Different letters indicate significant different ($p<0.05$) between the treatments in the same raw, ns= non significant

The changes of cell numbers of *Lb. pentosus* VM095, VM096, and YM122 during fermentation in soya milk are presented in Table 4.8. The cell numbers of *Lb. pentosus* in the soya milk fermented samples were significantly ($p<0.05$) increased

among strains during fermentation. At 6 h, the cell numbers for VM095, VM096, and YM122 reached to 9.34±0.06, 9.11±0.079, and 9.18±0.11 logCFU/mL, respectively. Maximum counts of cell numbers occurred at 18 h of fermentation for VM095 and YM122 (10.15±0.06 and 10.32±0.01 logCFU/mL) except for VM096 (9.41±0.10 logCFU/mL). The pH values declined from pH 6.35 initially to 5.31 at the 24 h fermentation. It is well known that lactobacilli have complex growth requirements. They require low oxygen tension, fermentable carbohydrates, proteins and their breakdown products, a number of B-complex vitamins, nucleic acid derivatives, unsaturated free fatty acids, and minerals such as magnesium, manganese and iron for their growth. Soya milk contains almost all that requirements, except iron as compared by MRS medium, which can be the reason for poor growth in soymilk than growth medium. In addition, soya milk is scarce in amino acids containing sulphur and this might be reason for their poor growth (Božanić et al., 2008). Wang et al, (2002 cited in Sumarna, 2008) reported that *Lb. delbrueckii* ssp. *bulgaricus* grew poorly in soya milk because they were not able to ferment sucrose and other soy carbohydrates.

In our finding, the tested strains in this study produced low amounts of lactic acid in soya milk and slightly grow up with cell numbers (7 upto 9 logCFU/mL) from the innitial of fermentation until 24 h. However, some LAB grew well in soya milk and produced less organic acids. The low levels of acid in soya milk presumably encouraged cell growth (Liu, 1997). On the other hand, Mital et al (1974) found that *Lb. acidophilus*, *Lb. cellobiosis*, *Lb. pantarum*, which utilised sucrose, could grew well and produced large amount of acid in soya milk. Soya milk was reported previously as a appropriate growth medium for some LAB such as *Lb. plantarum pentosus* SMN, 01*, Lb. plantarum* SMN, 25*, and Lb. plantarum pentosus* FNCC, 235 (Kamaly, 1997; Liu, 1997; and Sumarna, 2008).

4.6.2 Determination of pH value and titratable acidity

This study was carried out to determine the pH value and titratable acidity of soya milk fermented by 3 probiotic bacteria. The titratable acidity was calculated as percentage of lactic acid (w/v) of soya milk fermented. During fermentation of soya milk, the titratable acidity (%TA) increased from 0.12 ± 0.05 to 0.29 ± 0.03 depending on the strain used, meanwhile pH value decreased from 6.32 ± 0.02 to 5.34 ± 0.03 as displayed in Figure 4.10.

The pH values from VM095, VM096, and YM122 strains were not significantly ($p > 0.05$) among strains tested during 24 h fermentation. The initial pH values were 6.32 ± 0.02, 6.33 ± 0.02 and 6.32 ± 0.02, respectively. The pH values of those were decresed rapidly from 4 to10 h fermentation period from pH 6.20 to 5.30. It was observed that the texture of soya milk became slightly curding. At the end, the pH values in fermented soya milk by VM095, VM096, and YM122 strains were 5.31 ± 0.00, 5.37 ± 0.00, and 5.34 ± 0.01, respectively.

However, %TA in soya milk with VM095, VM096, and YM122 was not significantly ($p > 0.05$) among strains.The acidity was 0.12 ± 0.05% at the initial hour and increased rapidly within the 12 h to reach between 0.23 ± 0.04 to 0.26 ± 0.03%. At the same time of a slight increse of pH at 14 h, acidity also slightly droped, and then the development of acidity was increased slowly between 0.27 ± 0.00 to 0.29 ± 0.03% at 24 h fermentation. The highest acidity was observed in case of VM095 (0.29 ± 0.03%) at the end of 24 h fermentation. The pH values of soya milk fermented remained quite stable due to the buffering capacity of the soy protein (Itsaranuwat, 2003). The drop of pH and increasing of acidity was confimed by the growth with maximum changeable of cell number as displayed in Table 4.8.

In general, pH of soya milk dropped from 6.0 to 5.0 or below. Our results are similar to some profiles produced by LAB such as *Lb. plantarum* SMN, 25 and *Lb plantarum pentosus* FNCC,235 which took about 24 h of fermentation to reach pH 5.2 whereas lactic acid was found to be 1.2 mg/mL (Sumarna, 2008). In contrast, Bordignon et al (2004) reported that *Lb. casei* subsp. *casei* JCM 1134, *Lb. casei* subsp. *rhamnosus* IFO3425, and *Lb. delbrueckii* subsp. *bulgaricus* IFO13953 could grow well with lower pH between 4.0-4.74. Lowering pH of the culture and production of lactic acid are essential for soya milk fermented quality (Bordignon et al, 2004) because lactic acid is one of most important compounds in formation of flavor of fermentated products such as soya milk.

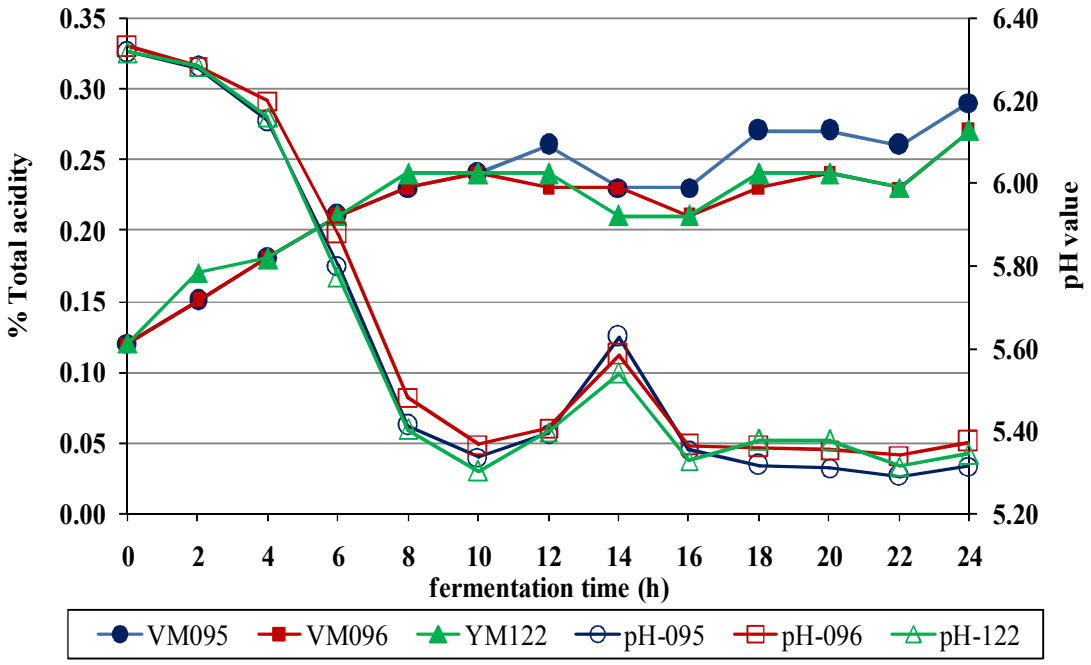

Figure 4.10 The pH change and acid production of *Lb. pentosus* strains VM095 (●=TA; ○= pH), VM096 (■= TA; □= pH), and YM122 (▲= TA; △= pH) in fermented soya milk

4.6.3 Determination of sugar contents in fermented soya milk

Soybean oligosaccharides have prebiotic effects and many reports have shown that their consumption confers to several health benefits, such as lowering of blood cholesterol, increased absorption of minerals, and prevention of some types of cancer (Roberfroid, 2007). However, one factor for the low consumer acceptability was the presence of high levels of non-digestible oligosaccharides. Stachyose and raffinose are the principal oligosaccharides in soya milk. They are believed to cause flatulence in human after eating soybean products. These sugar can be hydrolyzed by β-galactosidase. Several researches have reported that LAB can produce of galactosidase (Wang et al, 2003). *Lactobacilli* are also extensively used as probiotics. Soya milk has been examined as a substrate for the *Lactobacillus* species such as *Lb. casei*, *Lb. fermenti*, *Lb. fermentum*, *Lb. acidophilus* (Garro et al, 1999, 2004; Chumchuere and Robinson, 1999; Wang et al, 2002, 2003; Farnworth et al, 2007).

Changes of stachyose and raffinose in soya milk fermented by *Lb. pentosus* VM095, VM096, and YM122 are shown in Table 4.9. Regardless of *Lb. pentosus* strains used, levels of stachyose and raffinose slightly decreased as the fermentation

time increased, suggesting that all bacterial utilized these sugars. The strains VM096, YM122 and VM095 consumed stachyose significant different ($P < 0.05$) at the end of fermentation period by 47.88% (0.57 ± 0.05 to 0.30 ± 0.01 mg/mL), 37.74% (0.57 ± 0.03 to 0.35 ± 0.02 mg/mL) and 27.10 % (0.52 ± 0.02 to 0.38 ± 0.01 mg/mL) respectively. However, raffinose was reduced 44.50% (0.20 ± 0.02 to 0.11 ± 0.00 mg/mL), 40.56% (0.19 ± 0.05 to 0.11 ± 0.01 mg/mL) and 0% (0.12 ± 0.00 to 0.12 ± 0.00 mg/mL) respectivly. After 24 h, soya milk fermented with VM096 strain contained stachyose 0.30 ± 0.01 mg/mL. The levels were higher than those in soya milk fermented with YM122 and VM095. This showed that VM096 exploited these substrates more efficiently than YM122 and VM095.

Similar observations with soy oligosaccharide have been reported earlier, Chumchuere and Robinson (1999) observed the reduction of stachyose and raffinose levels in the fermented soya milk and they indicated that the utilisation of these sugars varied with the species of LAB. Wang et al (2003) reported that stachyose and raffinose in soya milk was utilized approximately 31.1–50.7% and 9.2–33.1% respectively by the single culture of LAB during a 24h fermentation period.

Bordignon *et al*. (2004) showed that raffinose, was substantially metabolized by LAB strains. The organisms in general metabolized stachyose by over 66% after 24 h with *Lb. plantarum SMN, 25,* and *Lb. plantarum pentosus SMN, 01* showing the highest hydrolysis of 78% and 72.5%, respectively. Moreover, Mital et al. (1974) demonstrated that fermentation of soya milk with lactic cultures possessing galactosidase activity reduced raffinose and stachyose contents.

HPLC analyses in Table 4.9 showed the sugar contents in soya milk fermented by *Lb. pentosus* VM095, VM096, and YM122. D (+) glucose, D (+) galactose, D (-) fructose and treharose contents were maintained from initial fermentation period until the end. These results demonstrated that *Lb. pentosus* strain could utilise stachyose and raffinose then breakdown into form monosaccharide and used various sugars to support their growth in soya milk fermention. On the other hand, the reduction in the content of stachyose, raffinose and an increase in the content of monosaccharide such as glucose, galactose was noted in Table 4.9. In fermented soya milk, the concentration of starchyose and raffinose decreased over time, the concentrations of D (+) glucose, and D (+) galactose in all the fermented milks increased. The D (-) fructose and treharose concentration in soya milk remained low during the 24 h fermentation. The sugar

utilisation results indicated that the 3 strains of *Lb. pentosus* have ability to use raffinose and monosaccharide such as glucose including another mono-sugar such as D (+) galactose, D(-) fructose, and treharose. This may be attributed to the hydrolysis of stachyose, raffinose during fermentation. Therefore, it is not surprising that a higher content of glucose plus galactose and fructose in soya milk cultured with LAB were observed (Chumchuere and Robinson, 1999; Wang et al, 2003; and Yang and Zang, 2009).

Table 4.9 Sugar contents in fermented soya milk by *Lb. pentosus* (VM095, VM096, YM122) strains for 24 h.

Incubation Time (h)	*Lb. pentosus* strains	Type of sugar components (mg/mL) in Soymilk fermentation								
		FOS	Maltotetraose	Starchyose	Raffinose	D(-)Maltose	Treharose	Lactose	Sucrose	D(+)Glucose
0	VM095	nd	nd	0.52±0.02ns	0.12±0.00ns	nd	0.04±0.01b	nd	nd	1.88±0.14ns
	VM096	nd	nd	0.57±0.05ns	0.20±0.02ns	nd	0.06±0.00a	nd	nd	2.02±0.17ns
	YM122	nd	nd	0.57±0.03ns	0.19±0.05ns	nd	0.05±0.01ab	nd	nd	2.20±0.29ns
6	VM095	nd	nd	0.48±0.02ns	0.12±0.01ns	nd	0.07±0.00	nd	nd	1.80±0.10ns
	VM096	nd	nd	0.49±0.01ns	0.19±0.05ns	nd	0.04±0.00	nd	nd	1.70±0.29ns
	YM122	nd	nd	0.51±0.04ns	0.14±0.02ns	nd	0.06±0.00	nd	nd	1.87±0.20ns
12	VM095	nd	nd	0.48±0.00ns	0.14±0.008ns	nd	0.04±0.00b	nd	nd	2.01±0.02b
	VM096	nd	nd	0.50±0.01ns	0.13±0.00ns	nd	0.04±0.00b	nd	nd	2.18±0.02a
	YM122	nd	nd	0.42±0.12ns	0.13±0.01ns	nd	0.06±0.00a	nd	nd	2.19±0.00a
24	VM095	nd	nd	0.38±0.01a	0.12±0.00ns	nd	0.06±0.01ns	nd	nd	2.34±0.11ns
	VM096	nd	nd	0.30±0.01b	0.11±0.01ns	nd	0.05±0.00ns	nd	nd	2.16±0.04ns
	YM122	nd	nd	0.35±0.02a	0.11±0.00ns	nd	0.06±0.00ns	nd	nd	2.23±0.09ns

Note: the meanvalues±SD in the same column, in the same time of each strain with different small letters were significant different ($p<0.05$), ns= non significant, nd= non detectable

Table 4.9 Continued

Incubation Time (h)	*Lb. pentosus* strains	Type of sugar components (mg/mL) in Soy milk fermentation									
		Myo-innositol	Mannose	D(+) Galactose	Xylose	D(-) Fructose	Mannitol	Sorbitol	L(-) Rhamnose	L(+) Arabinose	D(-) Arabinose
0	VM095	nd	nd	0.21±0.01ns	nd	0.04±0.01ns	nd	nd	nd	nd	nd
	VM096	nd	nd	0.18±0.04ns	nd	0.04±0.01ns	nd	nd	nd	nd	nd
	YM122	nd	nd	0.20±0.03ns	nd	0.04±0.00ns	nd	nd	nd	nd	nd
6	VM095	nd	nd	0.19±0.00ns	nd	0.03±0.00b	nd	nd	nd	nd	nd
	VM096	nd	nd	0.19±0.04ns	nd	0.06±0.03a	nd	nd	nd	nd	nd
	YM122	nd	nd	0.17±0.04ns	nd	0.07±0.03a	nd	nd	nd	nd	nd
12	VM095	nd	nd	0.20±0.00c	nd	0.04±0.00	nd	nd	nd	nd	nd
	VM096	nd	nd	0.23±0.00b	nd	nd	nd	nd	nd	nd	nd
	YM122	nd	nd	0.23±0.00a	nd	nd	nd	nd	nd	nd	nd
24	VM095	nd	nd	0.22±0.01ns	nd	0.05±0.00ns	nd	nd	nd	nd	nd
	VM096	nd	nd	0.23±0.01ns	nd	nd	nd	nd	nd	nd	nd
	YM122	nd	nd	0.22±0.00ns	nd	0.05±0.00ns	nd	nd	nd	nd	nd

Note: the meanvalues±SD in the same column, in the same time of each strain with different small letters were significant different ($p<0.05$), ns= non significant, nd= non detectable

4.6.4 SCFAs production in soya milk during fermentation

The amount of SCFAs was measured by HPLC using a LUNA C-18 column (4.6 x 250 mm id., 5µm) at 38 °C with UV detector and 10 mM $NaHPO_4$ buffer (pH 2.5) was used as the mobile phase at a flow rate of 1.0 mL/min. The lactic acid and SCFAs production in soya milk fermentation by *Lb. pentosus* VM095, VM096, and YM122 are presented in Table 4.10

Lactic acid levels in soya milk fermented by VM095 was higher significant different ($p < 0.05$) compared with YM122 and VM096 at 12 h. At the end, lactic acid arranged 0.59 ± 0.01 mg/mL. These results agree with the acidity values and pH changes (Figure 4.10) in fermented soya milk. In addition, the highest enumeration of *Lb. pentosus* strains in soya milk found at 12 h.

In case of SCFAs, acetic acid and propionic acid were decreased significant different ($p < 0.05$) over time fermentation 24 h, acetic acid decreased from to 9.59 ± 0.61 to 2.88 ± 0.07 µmol/mL (for YM122) and propionic acid decreased from 5.77 ± 0.68 to 3.38 ± 0.64 µmol/mL (for VM096). The concentration of the butyric acid and N-valeric acid were increased by time fermentation and iso-butyric acid was not detected the samples cultured by VM096 at the same time. At the end of fermentation, the concentration of acetic acid propionic acid, butyric acid, iso-butyric acid and n-valeric acid by VM095 had higher significant ($p < 0.05$) than VM096 and YM122 about 3.43 ± 0.08, 4.41 ± 0.39, 2.89 ± 0.25, 2.41 ± 0.05, and 28.80 ± 3.90 µmol/mL respectively. The results found that the concentration of starchyose and raffinose decrease over time. As sugar in soya milk was decreasing, the concentrations of lactic acid and some of SCFAs such as butyric, iso butyric, n-valeric acids in the fermented soya milks were increased. In our study iso-butyrate acid was identified only in some samples of soya milk fermented. Their concentrations depend on the cultured of strain used.

SCFAs are carboxylic acids with 1- 6 carbon atoms such as acetic, propionic and butyric acids are mainly formed during microbial fermentation of carbohydrate (Huda-Faujan et al, 2010). It may have specific roles, including beneficial health implications. Butyric acid was addressed to be more beneficial for promoting colonic health and more effective for stimulating the proliferation of intestinal mucosal cells than acetic and propionic acid (Henningsson et al. (2002). It also is the main energy substrate for the colonocytes and play an important role in the prevention of distal UC (Cummings, 1997), Crohn's disease, and cancer (Scheppach et al, 1995; Floch and

Hong-Curtiss, 2002) also, induce of tumor cell lines (Barnard and Warwick, 1993). In addition, SCFAs may have health-promoting effects, both locally in the colon and systemically, e.g. on glucose and cholesterol metabolism (Huda-Faujan et al, 2010).

Table 4.10 SCFAs production in fermented soya milk by *Lb. pentosus* (VM095, VM096, YM122) strains for 24 h.

Incubation Time (h)	*Lb. pentosus* strains	Type of SCFAs components (µmol/mL) in fermented soymilk					
		Lactic acid (mg/mL)	Acetic acid	Propionic acid	N-valeric acid	Butyric acid	Iso-butyric acid
0	VM095	0.42±0.01ns	7.71±0.31b	4.68±0.09ns	8.62±1.39c	0.64±0.00b	1.30±0.10b
	VM096	0.42±0.00ns	9.18±0.02a	5.77±0.68ns	22.02±2.41a	0.77±0.10b	nd
	YM122	0.45±0.01ns	9.59±0.61a	5.02±0.22ns	14.71±0.51b	1.41±0.17a	1.66±0.00a
6	VM095	0.65±0.01a	4.03±0.61b	2.76±0.05c	6.41±1.10b	0.43±0.00	2.13±0.00
	VM096	0.50±0.03b	5.18±0.40ab	4.38±0.07b	56.05±2.79a	0.43±0.00	0.00±0.00
	YM122	0.66±0.01a	5.97±0.01a	4.90±0.14a	61.09±4.69a	1.12±0.00	0.45±0.00
12	VM095	0.78±0.01a	3.54±0.33c	5.21±1.18ns	8.27±0.63c	1.42±0.09ns	0.98±0.10b
	VM096	0.70±0.01b	7.82±0.35a	4.97±0.10ns	14.26±2.30b	1.72±0.47ns	nd
	YM122	0.72±0.00b	4.69±0.21b	4.68±0.24ns	19.50±0.00a	1.64±0.30ns	1.51±0.06a
24	VM095	0.59±0.00ns	3.43±0.08ab	4.41±0.39ns	28.80±3.90a	2.89±0.25a	2.41±0.05a
	VM096	0.59±0.01ns	4.16±0.40a	3.83±0.64ns	24.62±3.21ab	2.43±0.21ab	nd
	YM122	0.59±0.01ns	2.88±0.07c	3.37±0.24ns	19.10±0.10b	2.06±0.17b	1.58±0.36b

Note: the meanvalues±SD in the same column, in the same time of each strain with different small letters were significant different ($p<0.05$)
ns= non significant, nd= non detectable

4.7 Soya beverage properties

Consumption of soybean as a food product is not very popular due to its off-flavor. The other reason is that some people feel uncomfortable upon consumption due to the presence of nondigestible oligosaccharides. To introduce soy-based products to people who are not familiar with its specific flavor and taste, it is required to develop a good flavoured soy-based food. Lactic acid fermentation would be a promising way to enhance soybean flavour because some LABs are effective to reduce the off-flavor of soybean. In addition, supplementation of soybean with flavouring or sweeteners is the other promising way to improve the products properties.

Some researchers have attempted to improve the properties of fermented milk products by addition of natural flavouring or sweeteners such as honey syrup, strawberry, corn syrup are some of the flavors that are quite acceptable. Its functional properties, honey has been gaining interest as a substitute flavouring and sweetener in foods such as yoghurt (Păucean et al., 2011; Roumyan et al., 1996; Chick, 2001). Honey is a rich source of carbohydrates (fructose, glucose, maltose, sucrose etc.). Its low pH value, due to a variety of organic acids, makes honey compatible with much food (Varga, 2006). The effect of honey addition on the 4 basic tastes (sweet, sour, bitter and salty taste). Its properties decreased the sourness of solutions and improved consumer acceptability of sour products, honey can be incorporated into fermented dairy products. Honey flavour is an important quality for its application in food industry and also a selection criterion for the consumer's choice (Bogdanov, 2008).

4.7.1 Sensory evaluation

The results summarised the sensory evaluation for appearance, color, odor, taste, mouth feel, and overall acceptance of fermented soya milk by 3 probiotic *Lb. pentosus* strains VM095, VM096, and YM122. The fermented soya milk without supplementation with 10% (w/v) honey syrup (SF095, SF096, and SF122) and fermented soya milk supplementation with 10% (w/v) honey syrup (SB095H, SB096H, and SB122H) are presented in Table 4.11. The perceived sample quality was represented by the mean score base on a 9-point hedonic scale range from dislike extremely (1) to like extremely (9) and the higher scores show the more desirable sample for panelists. Overall, the results showed that the score on odor, taste, mouth feel, and overall acceptance of SB095H, SB096H, and SB122H were significant ($p <$

0.05) higher in comparison with SF095, SF096, and SF122. The panelists could not detect any different in appearance and color among all samples. However, supplementation with honey syrup was also found to be as good as without honey syrup with the mean scores higher than 6 (like slightly). The mean scores of sensory evaluation for all attributes ranged between 3.10 ± 1.63 and 7.23 ± 1.31 representing the low-range to almost high range of the scale.

The supplementation of 10% (w/v) honey syrup in soya milk fermented showed a significant improvement of odor, taste, mouth feel compared with the samples without honey syrup. Overall acceptance were scored 4.5 (dislike slightly) for the samples without honey syrup whereas samples supplemented with 10% (w/v) honey syrup had significantly ($p < 0.05$) higher scores ranged between 6.93 ± 1.40 to 7.23 ± 1.31 which indicated 'like moderately'. In term of taste, the samples supplemented with 10% (w/v) honey syrup had significantly ($p < 0.05$) higher score ranged between 6.87 ± 1.62 to 7.05 ± 1.53 which indicated 'like moderately' in comparison with the samples without honey syrup which were scored 3.10 to 3.50 (dislike moderately). Odor was scored between 5.20 to 5.60 (neither like nor dislike) for samples without honey syrup while supplementation with 10% (w/v) honey syrup were significantly ($p < 0.05$) higher score ranged between 6.53 ± 1.83 (like slightly) to 7.00 ± 1.71 **(**like moderately). Mouth feel were scored at 5.40 to 5.50 (neither like nor dislike) for samples without honey syrup whereas supplementation with 10% (w/v) honey syrup were significantly ($p < 0.05$) higher scored between 6.83 ± 1.50 to 6.93 ± 1.49 (like moderately). Comparing the overall sensory results, samples supplementation with 10% (w/v) honey syrup showed a more promising soya beverage properties and sample SB095H was highest scored on appearance, color, odor, taste, mouth feel, and overall acceptance by 6.96 ± 1.52, 7.08 ± 1.52, 7.00 ± 1.71, 7.05 ± 1.53, 6.93 ± 1.49, and 7.23 ± 1.31, respectively.

The results obtained in this study indicated that the penelists reacted positively to the properties of soya milk added with honey syrup. The soya milk fermented had the taste and odor properties with the lowest mean score between of 3.10 to 3.53 and 5.20 to 5.60 respectively. The results suggested that soya milk fermented had the sharp/sour taste, flavor-off, which made it unfovourable for panelists. In general, fermentated milk products are characterized by lack of flavor because they possess an alcohol dehydrogenase, which converts acetaldehyde to ethanol (Itsaranuwat, 2003).

These results agreed with previous reports of Amiri (2010), studied on the symbiotic acidophilus milk prepared using starter culture (*Lb. acidophilus, B. bifidum* and *Lb. casei*) and prebiotic additives (oat, inulin, honey) singly or in combination. The results showed, sensory score significant increased with the colour, flavour, texture and overall acceptance of samples when added with inulin (10% w/v) or honey (7% w/v). Similar result was obtained by Riazi and Ziar (2012). They found that honey had a good effect on sensory properties of fermented milk with *bifidobacteria*. The points allocated for colour, body-texture and taste showed that increased in honey content brought about an improvement in the texture, flavour and aroma of the products ($p < 0.05$). On the other hand, Păucean et al. (2011) reported honey addition. They investigated that at the beginning of storage, taste and flavor intensity of kefir-type product has increased significantly ($p < 0.01$) with the honey's level addition. Panelists founded that kefir-type product with 1% (w/v) honey was weak in taste and flavour but the 4% (w/v) honey level was founded too sweet. The flavour intensity of the sample with 2.5% (w/v) added honey was considered optimium. The odour, the colour and the appearance values had no significant ($p > 0.01$) affected by honey addition.

Table 4.11 Comparative sensory evaluation of fermented soy milk (SF) with *Lb. pentosus* strains (VM095, VM096, and YM122), and soya beverage (SB) supplementation with 10% (w/v) honhey syrup (H).

Sample	Appearance^{ns}	color ^{ns}	odor	taste	Mouth-feel	Overall acceptance
SF095	6.58±1.65	7.10±1.45	5.60±1.86b	3.53±1.74b	5.40±1.88b	4.55±1.71b
SF096	6.75±1.64	7.08±1.42	5.20±1.74b	3.10±1.63b	5.48±1.77b	4.58±1.55b
SF122	6.63±1.56	7.00±1.60	5.38±1.73b	3.20±1.77b	5.50±1.77b	4.53±1.43b
SB095H	6.96±1.52	7.08±1.52	7.00±1.71a	7.05±1.53a	6.93±1.49a	7.23±1.31a
SB096H	6.50±1.78	6.53±1.77	6.60±1.69a	6.97±1.56a	6.90±1.43a	6.93±1.40a
SB122H	6.60±1.86	6.53±1.92	6.53±1.83a	6.87±1.62a	6.83±1.50a	6.93±1.61a

Note: Values are the mean ± SD within the same column followed by the different letter indicate significant differences (p<0.05) between the treatments, n = 40. 9= like extremely and 1= dislike extremely

- Soya milk fermented by *Lb. pentosus* VM096, VM096, and YM122 strain without 10% (w/v) honey syrup (coded; SF095, SF096, and SF122, respectively).

- Soya milk fermented by *Lb. pentosus* VM096, VM096, and YM122 strain supplementation with 10% (w/v) honey syrup were soya beverage (coded; SB095H, SB096H, and SB122H, respectively).

4.7.2 Survival of probiotic strains and pH change during storage periods

The survival of probiotic *Lb. pentosus* strains (VM095, VM096, and YM122) and changes of pH in soya beverage (SB095H; SB096H, and SB122H), fermented soya milk (SF095, SF096, and SF122) was investigated during storage 7 day intervals (0, 7, 14, 21, and 28) over 28 days in the refrigerator at 4 ^{o}C. Total colony counts using pour plate techinique and selective media (MRS agar) was used to determine change in viable counts. The changes in viable counts of VM095, VM096, and YM122 strains and pH changes during storage was showed in Table 4.12. The viable cells of *Lb. pentosus* VM095, VM096, and YM122 strain in soya beverage and fermented soya milk were significant ($p < 0.05$) increased. The highest survivals of cells were found in the 7-14 days and survival in soy beverage was higher than fermented soya milk. The result found that the viable cells of all strains were reached 10 logCFU/mL. The initial cell counts were 10.72-10.94 logCFU/mL. Cells was increased between 12.56-13.05 logCFU/mL in 7 days storage. In 21 days, an increasing in viable cells were found in all samples due to their have ability to utilise honey sugar (fructose, glucose, maltose, sucrose etc.) and soybean sugar especially galacto-oligosaccharide, whereas the latter microorganism lacks this ability (Itsaranuwat, 2003). Moreover, the various oligosaccharides found in honey may be responsible for enhanced the growth of some bacterial strains (*Lactobacillus, Streptococcus, Bifidobacterium)* (Bogdanov, 2008). The addition of honey to fermented soya milk was significant ($p < 0.05$) on the growth and survival of *Lb. pentosus* strains at 14 days storage. The final, 28 days storage found that the viable counts of all samples decreased as same as the cells at innitial due to the long time storage, as the storage time increased, the viable counts of bacterial decreased considerably (Sodini et al, 2002).

The pH values of soya beverage was lower than fermented soya milk as presented in Table 4.12. In the presence of honey, pH of SB095H, SB096H, and SB122H were significant different ($p < 0.05$) during storage at 4°C for 28 days. The pH values of SB095H, SB096H, and SB122H decreased from 4.77 ± 0.03 to 3.49 ± 0.02, 4.79 ± 0.02 to 3.64 ± 0.04, and 4.80 ± 0.03 to 3.64 ± 0.04, respectively. It was lower than fermented soya milk without honey due to variety of organic acids in honey (Varga, 2005).

The results supported by Bogdanov (2008), pH of milk products added with 1% and 2.5% honey were decreased by 0.22 and 0.19 units respectively. In any of the

studied levels 1% to 4%, the honey addition increased the viable counts of lactococci at all sampling intervals and bacteria were present at sufficiently high level by 7-8 logCFU/mL during the product shelf-life.

Table 4.12 Survival of *Lb. pentosus* strains in fermented soya milk and soya beverage added 10%(w/v) honey syrup.

Parameter	**Storage time (days)**	Survival of *Lb. pentosus* strains*					
		SF095	SF096	SF122	SB095H	SB096H	SB122H
Viable cell counts (log10 CFU/ mL)	0	10.80±0.06d	10.72±0.07e	10.94±0.04e	10.75±0.05e	10.81±0.09d	10.91±0.08c
	7	12.97±0.02a	12.92±0.08a	12.91±0.02a	12.56±0.07b	12.89±0.04b	13.05±0.04a
	14	12.70±0.08b	12.80±0.07b	12.68±0.07b	13.19±0.13a	13.27±0.03a	13.04±0.10a
	21	12.11±0.14c	12.07±0.03c	12.02±0.04c	12.23±0.04c	12.16±0.11c	12.19±0.06b
	28	10.89±0.08d	11.22±0.06d	11.39±0.03d	11.14±0.12d	10.60±0.07e	10.56±0.06d
pH values	0	5.28±0.03a	5.32±0.04a	5.32±0.02a	4.77±0.03a	4.79±0.02a	4.80±0.03a
	7	4.91±0.02b	4.90±0.01b	4.90±0.01b	3.75±0.03b	3.89±0.04b	3.87±0.02b
	14	4.90±0.01b	4.88±0.02b	4.90±0.01b	3.69±0.01c	3.84±0.02c	3.84±0.04b
	21	4.89±0.01b	4.88±0.02b	4.89±0.02b	3.59±0.01d	3.78±0.01d	3.75±0.03c
	28	4.82±0.02c	4.81±0.01c	4.85±0.02c	3.49±0.02e	3.64±0.04e	3.64±0.04d

Note: Values were Means ± SD from 3 replication. Different letters that followed numbers within the same column in the same parameters indicated significant different (p<0.05) between the treatments. Storage in a refrigerator at 4 ^{o}C for 28 days. *SF095, SF096, and SF122 = cultured with VM096, VM096, and YM122 strain without 10% (w/v) honey syrup. *SB095H, SB096H, and SB122H = cultured with VM096, VM096, and YM122 strain supplementation with 10% (w/v) honey syrup.