CHAPTER 5

CONCLUSION

The aims of this study were to: (1) Investigate health-promoting properties of the probiotic *Lb. pentosus* strains (2) produce a probiotic soya beverage and study its properties, evaluate its sensory properties, and product's shelf life. To determine the growth profile and pH changes in MRS medium contain different sugar followed by determination of sugar utilisation and SCFAs production by HPLC technique. Then, health-promoting properties were determined such as screening BSH activity and *in vitro* cholesterol binding activity, ZEA binding ability, and the *in vitro* adhesion ability by cell surface hydrophobicity. Then, the tested strains were selected to be inoculants for soya milk fermentation and produce a probiotic soya beverage. The fermented soya milk properties was investigated by enumeration of tested strains, acidity and pH values, sugar utilisation and SCFAs production. Study on soya beverage properties, sensory evaluation test and survival of probiotic during storage 28 days were applied. Finally the following results were obtained;

(1) The growth, sugar utilisation and SCFAs production of *Lb. pentosus* in MRS media were dependent on type of sugar present as well as the strain of *Lb. pentosus.* Five of eight tested strains (UM054, UM055, VM095, VM096 and YM122) had ability to growth in oligosaccharide (lactose, raffinose and FOS-MRS) better than monosaccharide (glucose). The maximum growth at 12-15 h found in oligosaccharide sugars, reached 2.72-2.91, whereas OD in glucose 2.54-2.62. The pH values at 24 h were achived 3.60-3.70. On the other hand, all strains had a higher capacity utilisation of monosaccharide than oligosaccharide (significant different). The consumption of glucose (94.83 %) greater than raffinose (68.78%), lactose (68.34 %), and FOS (22.65 %). However, this study found those 5 strains were expressed the capability to produce organic acid and SCFAs were lactic acid (16.64-18.13 mg/mL), acetic acid (89.02-98.49 µmol/mL), propionic acid (38.31-64.70 µmol/mL), butyric acid (37.82-46.08 µmol/mL), Therefore, hightlight of probiotic selection focusing on probiotic properties. Oligosaccharide is prebiotic sugars and it can be used as a carbon source to promote the growth of probiotic *Lb. pentosus* strains and the main end-products from carbohydrate fermentation by probiotic are SCFAs which can contribute to health benefits.

(2) For BSH activity and *in vitro* cholesterol binding activity, 8 *Lb. pentosus* strains exhibited BSH activities as demonstrated by precipitation zones ($p < 0.05$) with diameters between 8.83 mm to 10.17 mm. The greatest precipitation zone was found in VM 096 followed by VM095, YM122, respectively. The amount of cholesterol reduced ranged 20.31 to 31.30 μg/mL (29.01 to 44.71 % reduction). All strains showed a significant ($p < 0.05$) reduction in cholesterol concentration in culture broth. The highest percentage of cholesterol reduced was VM096 follow by UM055, VM095, YM122, respectively. In this study, the VM096, VM095, and YM122 strains have shown moderate to good for the cholesterol reducing activity relate by BSH enzyme activity from culture media. Those of 3 strains exhibited to decrease cholesterol from media. *lactobacillus* species were able to reduce cholesterol *in-vitro* via several mechanisms such as an uptake or assimilation of cholesterol, cholesterol adherence to cells wall or its incorporation into cells, also bile salt deconjugation (Liong and Shah, 2005; Silirun, 2010). Reduction of cholesterol, in the added cholesterol media is considered as an indication for the selection of probiotic strains with cholesterol assimilation property (Gilliland and Walker, 1990; Lin and Chen, 2000).

(3) *In-vitro* testing of probiotic 8 *Lb. pentosus* strains for ZEA binding and adhesion ability by cell surface hydrophobicity confirmed their health-promoting properties. The binding abilities of 8 tested strains showed significant ($p<0.05$) at various concentration levels of ZEA in buffer solution. At the highest concentration level of ZEA (75.70 μg/mL), all strains could bind ZEA in between 44.93 to 62.12 μg/mL (60.15 % to 83.17%), 3 strains that could greatest detoxify ZEA higher than 80% was JM0812 followed by UM054 and UM055. Never the less, testing at the lower level of ZEA concentration (1.10-23.08 μg/mL), the best strain could eliminate < 50% ZEA. This results supported by Joannis-cassan et al (2011), the level of adsorbent 5.0 μg/mL was sufficient to bind at least 20% of toxins. However, *Lb. rhamnosus GG* and *Lb. rhamnosus* LC-705 had ability to bind ZEA in liquid medium 38% and 46%, respectively (El-Nezami et al., 2002). ZEA is 1 of 5 mycotoxins which are considered to be important in human health and causing economic losses by contaminated in various cereals crop such as soybean, rice, corn, wheat and grain crops (Yazar and Omurtag, 2008; Zinedine et al. 2007) also indirect source of exposure for humans and significant hazard to food chain. The contamination of ZEA showed highest in north Asia (63%), Southeast Asia was second (37%), and South Asia (4%) of positive samples. However, the average amount of ZEA contaminate in Asia was 129 mg/mL, which was within range of the maximum limits

regulated in Asian countries (Anukul et al, 2013). ZEA contamination in Thailand had few informations than other mycotoxins such as AFB1 and OTA. Although, the method to reduce ZEA contamination have been considerable attention but several safe and efficient methods are not practical and too expensive. Therefore, these study of binding ZEA efficiency by probiotic *Lb. pentosus* strains in our study is a first report in Thailand.
For cell surface hydrophobicity test, VM096 shown the highest value follow by UM055 and UM054 while, DM068, JM0812, and JM085 were indicated of low value. The cell surface hydrophobicity values were range 6.24% to 8.20% and had significant ($p<0.05$) among the tested strains. Meanwhile, probiotic which had the high adhesion ability, it possible to colonize and modulate the host immune system. *Lactobacillus* strain has beneficial health effects in humans by the protection against toxins contained in foods (Ouwehand et al., 2002; Saxelin et al., 2005; Fuchs et al., 2008). Cell hydrophobicity could indicate importance for bacterial cells adhesion and maintenance in the human gastrointestinal tract.

Based on the growth profile, sugar utilisation and health-promoting properties of the probiotic *Lb. pentosus* strains from this study, 3 strains were selected to be inoculants for soya milk fermentation. The VM095, VM096, and YM122 were selected to be a starter culture due to the strains were efficiency to use of soy oligosaccharide with exhibited a good growth profile and SCFAs production including the efficient probiotics for health benefit promoting. It could be provide the health benefit promoting on the consumer or provide the potential starter culture of fermented foods or beverages. All these reason will lead to select those strains to be the starter for produce the soya beverages.

(4) Three samples of fermented soya milk which single culture inoculated by VM095, VM096, and YM122 were determined. %TA, pH value had no significant while, maximum increased of cells number greater than 9 log CFU/mL and showed significant ($p<0.05$). The pH values in fermented soya milk arranged 5.31- 5.37 and the acidity between 0.27-0.293%. The VM096 strain exploited these substrates more efficiently than YM122 and VM095. As stachyose and raffinose were decreasing whereas, the concentrations of D (+) glucose, and D(+) galactose in all the fermenting milks increased. In case of lactic acid and SCFAs production, VM095 was significant ($p<0.05$) higher level than YM122 and VM096 From this results confirmed that the fermented soya milk with 3 of *Lb. pentosus* strains could provide the potential health-promoting properties effect on consumer. Thus, all these will produce the soya beverage with improved it properties for consumer acceptance.

(5) The soya beverage products were considered using the 9-point hedonic scale by the 40 tasters. The supplementation 10% (w/v) of honey syrup showed significant ($p<0.05$) higher acceptance score (odor, taste, mouth feel and overall) and viable cells than the samples without honey syrup. The soya beverage inoculated with VM095 strain had the best properties of sensory score and the viable cells reached the maximum to 10 log CFU/mL, pH value 3.49±0.02 store 4 ^{o}C for 28 days storage. This study confirmed of *Lb. pentosus* had ability to growth in soya beverage during storage to maintain high amount of viable cells relate to the suitable dosage used of probiotic microbes recommended at least 6 log CFU/mL of viable cells. Moreover, the starter culture properties and honey syrup with health promoting are usefulness. Therefore, these soya beverage products are very sufficient and suitable for health conscious consumers, vegetarians, and lactose-intolerant consumers.